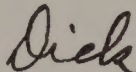
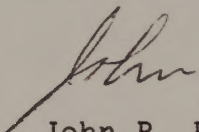


- b. Will an attractant lure parthenogenetic females to SWASS or traps?
- c. Will parthenogenetic females lay enough fertile eggs to become a serious problem?
- d. Is parthenogenesis in screwworms facultative or obligatory?
- e. If parthenogenetic females were to become a serious problem not controllable by present methods, is there an alternative biological control available?

More details will be in our forthcoming annual report of our Cooperative Agreement, but if you have any questions or need more details, please call.

Sincerely,


R. H. Richardson

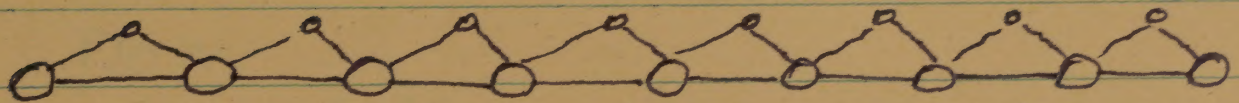

John R. Ellison

cc:
Dr. Percy Turner, Water Valley, Texas

Nov. 9, 1981

attractants —○—◇—○—

(11) Jack Plummer, —, consult ref.
diffusion of lepidopteran
pheromones



Improved oviposition attractant

→ Incidence + population dynamics
of the screwworm in S. Mex. including
Yucatan.



Improve insecticides —

—//—

Bait stations —

—//—

proper use of SWASS — dosage
+ prolong effectiveness

larval diet

adult diet

—//—

Participants -

Bram

Leo

Whitten

OHG

EAT

Dom

Bob Hoffman

Turner

Richardson

Ellison

✓ Arerhoff

✓ Meyer

F. Smith

✓ C. Hofmann

✓ R. M. Vargan

Bushland

✓ Meadows (2)

✓ Pineda -

Rm

131

6 pm

Fargo -

1. Support to the APHIS program will be both direct + indirect -

2. attractants -

simulate oviposition and feeding sites -

Coop. Agreem. NDSU, 4 yr.
\$120,000 -

Bacteria - microflora of the wound - Hammach + Gessner -

a. purification of bacterial products

b. genetic variability of a Proteus sp.

cx bioassay - using gravid females

— # —

a genetic sexing mechanism -

DDT, dieldrin, propoxur (Baygon)

— # —

Leo - There are likely to be population differences, not necessarily detectable by barytype analysis -

study of polytene chromosomes,
so far non-productive for screwworms -
recommend a 2-year study,
undertaken by a post-doc. -
or perhaps behavioral differences

utilization of Resources

1. Strain development

a. McInnis	5%	}	2 SY
b. Peterson	20%		
c. Ocampo	75%		
d. Vazquez	100%		

2. SWASS - Swormlure enhancement

a. Brown	10%	}	3.3 SY
b. Thompson	100%		
c. Macksley	75		
d. Peterson	50		
e. _____	100		

3. Genetics Res. (Population characteristics)

a. McInnis	95%	}	3.75Y
b. Whitten	75%		
c. Mangan	100%		
d. Post-doc (Fargo)	100%		

4. Other Attractants

a. Hammack	100	}	3.0 SY
b. Whitten	25		
c. Ring	100		
d. Macksley	25		
e. Graham	50		

5. Field Biology & Ecology

a. Spencer	100%	}	2.8 SY
b. Peterson	30%		
c. Brenner	100%		
d. Graham	50%		

15 SY

Nov. 10, 1981

presented by Dick Richardson

① Advantages of arraga -

X a highly diverse region genetically -
X some of the gamodemes in arraga
are also found in Central Mexico -
X discussion of "gamodemes" -
X several new warm gamodemes will
probably later be described by the
taxonomists as species -

X significance to the eradication
program:

X the "species" (new species) might
X or might not be significant to
X the eradication program but based
X on the arraga test we now
X know that this diversity of
species is significant to the
program -

Our clue in 1978 was the
failure of 009 in Arizona -

if heterozygotes from genetically
different parents can be identified
then gamodemes can be identified -

Oddly enough heterozygotes
can more easily be found when
a rare type and a common type
exist sympatrically -

Rodolfo Pavan -

Campinas, S.P., Brazil

(2)

Both V-81 and RGM-6 were largely type F -

test was designed to release small numbers - test the mismatch and the numbers needed to offset the mismatch -

comments on Fig. 1, p.5

with a stable population of 100 flies/mi², 25 ~~flies~~ sterile flies per mi² will eliminate the wild population - a theory obtained ~~from~~ ^{by} mathematical calculation -

Knipling's calculations are based on expanding populations not equilibrium populations -

~~Proposal~~

a large amount of additional analysis is needed before publication takes place -

a further search for diversity is needed - e.g. Pacific Coast between Chaparral and Sinaloa
also continue to screen for parthenogenesis, either facultative or obligatory -

(3)

Part 3 - increase sensitivity of the analysis by improving barytyping Q-banding as used in primate barytyping -

Part 4 - ageing - ~~has~~ studying apodemes

loan of a scope to John Gold is recommended — UV fluorescence — new objectives are recently marketed

Survey — ARS cooperation would be needed — trailer-lab + 2 technicians —

UT would have a person at Tuxtla to handle F-12

—//—

Part 1 — Completion of analysis — completion of electrophoretic analysis — subtle differences in the gels have indicated barytypic differences — subgroups within a single gamodeme might be identified —

John Ellison: Aruaga test was unique, a large amount of material was collected, a great deal can be learned by completing the analysis —

(4)

Part 2 - presumes use of certain
~~top~~ ARS capabilities at Tuxtepec and
Fargo

Post-doc. - about \$1200 per month

Part 3 - new approaches to genetic
analysis - new measurements
would be fed to a computer
DNA, also special types of
DNA, would be assayed - - -
semi-automation of karyotypic
analysis would be achieved
preparations would be fitted
into special pigeon-holes, if they
didn't fit they would be discarded
or re-examined by the cytologist -
no risk involved in the
measurement, this has been established -
- - - operate with 2% accuracy

Part 4 - - - Age determination -
Spencer Johnston, Tex. A + M -

Ken Castleman paper on
Drosophila

OUTLINE

I. Strategic Considerations

A. Basic assumptions.

B. Planning and preparation.

II

5

Nov. 10, 1981

1:30pm - Discussion of the UT program -

Boram : recapitulation of the ARS
program

Turner said

⌊ ARS scientists have not validated the UT hypothesis but it can be validated.

La Chance -

The UT group has started from a logical base; in the geographic range of the species differences in population are to be expected. Chromosomal polymorphism is well known in many species.

Parthenogenesis

merit of the proposal
group discussion on the use
of other means of detecting parthenogenesis

Natural Hybrids -

McJannet has recognized hetero-
zygotes

Electrophoretic Analysis

Whitten: Large sample sizes are needed

6

Completion of the analysis of
Arriaga material

LaChance —

research proposals from
the academic community as a
whole should be invited; proposals
should be directed toward the
development of new methods of
characterizing screwworm populations
in nature.

John Gold  

Bram — should we look for an
outside (cytological) geneticist who
could resolve the differences of
opinion



M. Vargas —

V-81 vs. RGM-6 in
the field, question to McInnis —

If a new strain is hard to
rear it doesn't matter that it is
effective in the field —

Vargas also supports the
idea that more research should
be done —

7

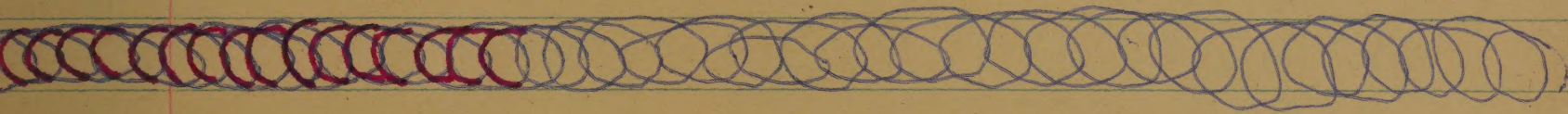
Bushland -



a. Curacao - accompl. w/ 800 flies
per mi^2

b. for the Florida program - 1000/ mi^2

c. early on in Texas - also 1000/ mi^2
and ^{virtual} eradication was achieved in 1964.





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Staff

RECEIVED NOV 30 1981

Beltsville, Maryland
20705

November 20, 1981

SUBJECT: Review of Research Proposal From the University of Texas -
Genetic Differences Among Screwworm Populations

TO: O. H. Graham, Project Leader
Screwworm Research Laboratory, Mission, TX

Subject research proposal from Dr. R. H. Richardson and Dr. J. R. Ellison, which was reviewed at a meeting in Mission, Texas, on November 10, 1981, consisted of four parts: (1) Completion of the genetic analysis of the Arriaga test (\$131,809); (2) Survey of genetic diversity in southern Mexico (\$27,130); (3) Improved methodology of data acquisition and analysis (\$61,990); and (4) Development of apodeme age and sterility determination (\$5,162).

I recommend that no further consideration be given to providing support for parts 1, 2, and 3 of the proposal. Should resources at the Screwworm Research Project, Mission, Texas, permit, I would recommend that favorable consideration be given to providing support for part 4 of the proposal (Development of apodeme age and sterility determination).

Since the question of reproductive isolation could be of critical importance to the further success of the screwworm eradication program, I wish to establish an ad hoc advisory committee as soon as possible to provide me and line managers with recommendations concerning the need for and types of research necessary to resolve this question. I would anticipate that the ad hoc committee will consist of three geneticists (one of whom to be identified by the University of Texas). I will work with you and Dr. Leo LaChance to organize this ad hoc committee and prepare its terms of reference.

RALPH A. BRAM
National Research Program Leader
Insects Affecting Man & Animals

cc:

Meeting Participants

T. B. Kinney
T. J. Army
H. G. Purchase
E. L. Kendrick
J. R. Johnston
C. H. Schmidt

GENETIC DIFFERENCES AMONG SCREWORM POPULATIONS
REVIEW OF RESEARCH PLAN
Mission, Texas
November 10, 1981

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Edgar A. Taylor	USDA, ARS, Mission, TX
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John Ellison	Zoology Dept., U of TX, Austin
Dick Richardson	Zoology Dept., U of TX, Austin
Dennis Ling	USDA, ARS, C/A, TX A&M, Mission, TX
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Moises Vargas Teran	Joint Com. Mexico City
Bud Turner	SWAHRF, NWG & NCA
Nazario Pineda	Joint Com. Mexico City
Leo LaChance	USDA, ARS, Fargo, ND
O. H. Graham	USDA, ARS, Mission, TX



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Beltsville, Maryland
20705

RECEIVED NOV 27 1981

Graham

November 23, 1981

SUBJECT: Trip Report -- Screwworm Research Project

TO: H. G. Purchase
Acting Chief, LVS

Location: Mission, Texas

Date: November 9-10, 1981

Objectives: To review 1982 screwworm research plans for Fargo, North Dakota; Mission, Texas; and Tuxtla Gutierrez, Mexico; to review an unsolicited research proposal from the University of Texas entitled, "Genetic Differences Among Screwworm Populations."

Results: The following screwworm research priorities were established for 1982:

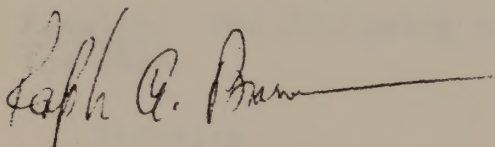
<u>Priority</u>	<u>Activity</u>	<u>SY's</u>
1	Strain development	2.0
2	SWASS-Swormlure enhancement	3.3
3	Genetics (population characterization and sexing)	3.7
4	Other attractants (including oviposition attractants, feeding attractants, etc.)	3.0
5	Field biology-ecology	2.8

In estimating the distribution of financial resources necessary to accomplish the above priority research activities, it was determined that approximately one-fourth of anticipated FY 82 gross funds or \$250,000 be allocated to the screwworm Research Project, Metabolism and Radiation Research Laboratory, Fargo, North Dakota, with the remainder being distributed to the Screwworm Research Project, Mission, Texas/Tuxtla Gutierrez, Mexico.

Following a review of the V-81 screwworm strain field evaluation data, ARS scientists recommended to APHIS that this strain be entered into production during the next strain change at the screwworm production facility at Tuxtla Gutierrez, Mexico. Work will continue to develop the "Arriaga Strain" as a backup by April 1, 1982.

During discussions of the development of a "male-only" screwworm strain, the question of field evaluating hand-sorted males, females, and males plus females was addressed. Recognizing the high cost and extreme stress that a field test of this magnitude would have on the limited manpower available in Mexico, it was recommended that a field test be deferred until results of research at Fargo provide a strong indication of the feasibility of developing a "male-only" strain.

November 10, 1981, was devoted to a review of an unsolicited research proposal from the University of Texas entitled "Genetic Differences Among Screwworm Populations." Representatives of ARS, APHIS, and the Mexico/American Screwworm Commission participated in a thorough review and discussion (including a review of previous research by the University of Texas group) of the proposal presented by Dr. R. H. Richardson and Dr. J. R. Ellison. I have recommended to the Screwworm Project Leader, Dr. O. H. Graham, that no further consideration be given to providing support for parts 1, 2, and 3 of the proposal. Should resources at the Screwworm Research Project, Mission, Texas, permit, I have recommended that favorable consideration be given to providing support for part 4 of the proposal (Development of apodeme age and sterility determination). In addition, I am taking steps to establish an ad hoc advisory committee to provide me and line managers with recommendations on the need for and types of research necessary to resolve the question of reproductive isolation, which could be of critical importance to the further success of the screwworm eradication program.



RALPH A. BRAM
National Research Program Leader
Insects Affecting Man & Animals

cc:

T. J. Army
E. L. Kendrick
J. R. Johnston
✓ O. H. Graham
P. J. Fitzgerald
C. H. Schmidt
L. E. LaChance
C. J. Whitten



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Beltsville, Maryland
20705

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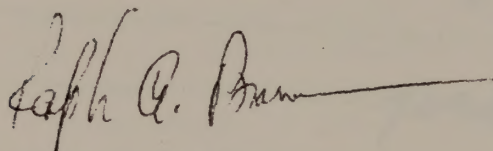
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RALPH A. BRAM
National Research Program Leader
Insects Affecting Man & Animals

cc:

T. J. Army
E. L. Kendrick
J. R. Johnston
✓ O. H. Graham
P. J. Fitzgerald
C. H. Schmidt
L. E. LaChance
C. J. Whitten

tel. conversation w/ Bram
12/08/81

After funds are distributed & if
enough money is available,

write to Richardson

offering to fund
proposal no. 4

tel. conversation w/ Brown
12/08/81

After funds are distributed to it
enough money is available

after-

====
= Codley =
=====

Robert R. (==)
leave Saturday



United States
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National
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Staff

Graham
Beltsville, Maryland
20705

RECEIVED DEC 18 1981

December 9, 1981

SUBJECT: Review of Research Proposal from the University of Texas -
Genetic Differences among Screwworm Populations

TO: T. B. Kinney, Jr.
Acting Administrator, ARS

THROUGH: T. J. Army
Deputy Administrator, NPS

H. G. Purchase
Acting Chief, LVS

In accordance with your telephone request of December 9, I have thoroughly reassessed the review of subject proposal and my recommendation to Dr. O. H. Graham that no further consideration be given to providing support for parts 1, 2, and 3 of the proposal. As a result of this reassessment, I stand firmly behind the recommendation with the confidence that it is based on the best available scientific judgement.

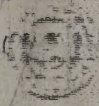
Drs. Richardson and Ellison were provided an opportunity to present not only a written report of their previous research on genetic differences among screwworm populations and proposed research for 1982, but also an entire morning was devoted to an oral presentation by these scientists to an eminent group of ARS scientists, APHIS screwworm program officials, and Mexico-American Screwworm Commission officials (list of participants enclosed). An afternoon session was spent in the review and analysis of the previous and proposed research. My recommendation, therefore, is based on the considered opinions of scientists whose credibility and scientific excellence are beyond question and whose basic interest is the continued success of one of the most important USDA programs protecting livestock in the United States.

RALPH A. BRAM
National Research Program Leader
Insects Affecting Man & Animals

Enclosure

GENETIC DIFFERENCES AMONG SCREWORM POPULATIONS
REVIEW OF RESEARCH PLAN
Mission, Texas
November 10, 1981

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Nazario Pineda	Joint Com. Mexico City
Leo LaChance	USDA, ARS, Fargo, ND
O. H. Graham	USDA, ARS, Mission, TX



RECEIVED DEC 16 1981

DEC 14 1981

SUBJECT: Review of Research at University of Texas

TO: K. Bran, NPS

Attached is a communication from Professors Richardson and Ellison on this topic. I asked them, through Dr. Turner, to provide me in writing with any concerns they had about the review. Please use the information they have provided as a basis for evaluating the review process and the resulting recommendations. You should communicate with any members of the review team as appropriate.

I will want, at the earliest possible time, your advice regarding the review, resulting recommendations and any additional action you deem necessary or appropriate.

T. B. Kinney, Jr.

T. B. KINNEY, JR.
Acting Administrator

Enclosure

ARS FACSIMILE MESSAGE

FROM: ARS
JKT: NO:

DATE: 12/15/81
TIME:

DEC 14 1981

Dr. R. B. Richardson
The University of Texas at Austin
Department of Zoology
Austin, Texas 78712

Dr. J. R. Ellison
The University of Texas at Austin
Department of Zoology
Austin, Texas 78712

Dear Professors Richardson and Ellison:

This is in response to your letter of December 3, 1981, regarding the review of the seromorph research that we have been funding at The University of Texas. I appreciate you taking the time to put your concerns in writing. I intend to ask Dr. Bram to consider the purpose and conduct of the review and let me know whether he or other reviewers recommend additional consideration. We cannot assume that there will be another review. I should also clarify why the list of suggested peer reviewers was asked for. When I talked with Dr. Turner, he indicated that you were not satisfied with the peer reviewers who were present at the review. I asked him who would have been acceptable and he cited a couple of the persons you have listed.

Centlemen, we must also keep in mind that a major purpose of the review was to contribute information that would allow us to make a decision as to whether we would continue support of the research if funds were available. We were not in a position of holding funds and asking if we should use them to continue this program.

As soon as I have received recommendations from Dr. Bram, I will be in touch with you.

Sincerely,

F. B. Ramsey, Jr.

F. B. RAMSEY, JR.
Academy Administrator

cc:

R. Bram w/copy of incoming

page 3 of 6

December 5, 1981

Dr. T. B. Kinney
Room 302A
Administration Bldg.
USDA
Washington, D. C. 20250

Dear Dr. Kinney:

In regard to the subject of peer review, I think it only prudent to review the work of Dr. McInnis along with that of the University of Texas group since he is the ARS geneticist actively engaged in field decisions in matters relating to genetics and strain selections.

Although Dr. Bram's desire to establish an ad hoc advisory committee to make recommendations concerning the question of reproductive isolation indicates his concern for this subject, I would like to additionally state that Dr. Hazzario Pinesca urged funding of the U T projects and Dr. Norvan Meyer was very interested in possible economies that might result from their research.

Sincerely,

Percy R. Turner



page 4 of 6

THE UNIVERSITY OF TEXAS AT AUSTIN
AUSTIN, TEXAS 78712

Department of Zoology

December 3, 1981

Dr. T. B. Kinney
Room 302A
Administration Bldg.
USDA
Washington, D. C. 20250

via Dr. Percy Turner

Dear Dr. Kinney:

With respect to your request through Dr. Percy Turner that we respond to the November 20, 1981 memorandum from Dr. Ralph Bram to Dr. Q. H. Graham, we were disappointed that a decision was reached to give "no further consideration" to support the completion of our screwworm research. It is especially distressing that the decision was reached by a group that contained no population or cytogeneticist by training or experience. It was our understanding after the review, which was conducted in our absence, that a review would be conducted by a 3-member panel of experts in the field. This review would include all of the genetic research on screwworms, including that conducted by ARS staff, and a recommendation would then be a basis for further funding. We enthusiastically supported such a detailed and comprehensive review, and have recommended it in the past.

There were two individuals at the review with credentials close to our work. Dr. Leo LaChance, who commented that he was not qualified to evaluate the findings, we understand suggested the outside review. Dr. Don McInnis, who is trained as an "ecological geneticist" according to his major professor, spent only two days in Austin learning the cytological techniques we use. The remainder of the group was comprised of approximately 7 entomologists and 5 veterinarians.

Particularly because our work has been a controversial issue, it is very important to have a highly competent, impartial panel evaluate the soundness of our results and interpretations, and relate them to the ~~state of the scientific literature~~ desirable that such a panel be of international renown, since this program is a model effort in biological control, and other programs will be planned after it is carefully and critically examined.

The features of our work and techniques that require special attention for selecting technically appropriate reviewers include:

1. Use of metaphase chromosomes to characterize species

research interests in the past several years has moved away from work related to natural populations. Wallace has worked with closely related species and speciation and is an active experimentalist and theoretician (as opposed to administrative scientist). His background is best for evaluating competitive and adaptive implications of a complex population, as well as broader aspects of population genetics and ecology.

Dr. Charles Michener, Dept. of Entomology, Univ. of Kansas, Lawrence, Kan. Dr. Michener, a member of the National Academy of Science, better covers general entomology and behavioral aspects of species isolation than those above, and is slightly less of a population geneticist than Wallace. He is not a cytogeneticist. His experience in taxonomy and identification of closely related species is widely respected. Michener's work with bees, particularly the "Brazilian" bee, and natural population dynamics when disturbances are involved is his forte in this group.

There are others mostly of a younger group and thereby somewhat lesser lights that we could name, but the above are our first choices in the various areas outlined.

There are many aspects of the genetic diversity of screwworms that have a critical bearing on the success and economy of the control program. The presence of more than one species in wild populations (see manuscript to appear in Science in January) precludes a continuation of the same procedures as have been used in the past. If diversity includes parthenogenetic forms, then the long term success of the program depends on the identification of such types, and the development of control procedures prior to their rise to levels of economic significance (see copy of letter to Dr. Graham, September 23, 1981). Economical and efficient completion of the program requires consideration of the genetic diversity, and we believe a considerable reduction in cost is possible as a result of increasing efficiency.

We understand that plans are being developed for regular review of ARS CRIS projects by peer review. We believe that many advantages would be derived from this policy. From our experience, one of the major advantages would be avoiding decisions being based on grossly inadequate evaluation, a dangerous course of action considering the crucial role played in the agricultural economy by research directly funded by the USDA.

R. H. Richardson

Professor

John A. Ellison



United States
Department of
Agriculture

Agricultural
Research
Service

National
Program
Staff

Beltsville, Maryland
20705

RECEIVED DEC 21 1981

Graham

December 17, 1981

SUBJECT: Review of Research at University of Texas

TO: T. B. Kinney, Jr.
Acting Administrator, ARS

In response to your memorandum on this subject of December 14, I have thoroughly reassessed the review process of Drs. Richardson's and Ellison's proposal for screwworm research and the resultant recommendations which I made to the Screwworm Project Leader, Dr. O. H. Graham. In making this reassessment, I have contacted appropriate scientists who participated in the review process and have reevaluated all documentation associated with the exercise of which I was chairman. The review process was conducted in an open and impartial manner and the conclusions from which recommendations resulted were based on the best available scientific judgement. The ARS and APHIS participants in the review, whom I have since contacted, unanimously support my original recommendations.

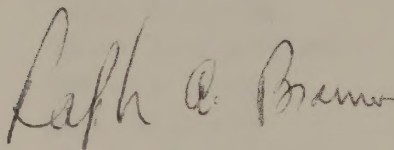
As a result, I can only reiterate my recommendations that no further consideration be given by ARS to providing support for parts 1, 2, and 3 of the proposal and that, should resources permit, favorable consideration be given to providing support for part 4 (Development of apodeme age and sterility determination). In addition, I continue to feel that a technical committee should be established to address the question of possible reproductive isolation in screwworm populations. I would expect that this technical committee will deal with the scientific question of reproductive isolation in all its aspects, including future avenues of research; it, would not merely evaluate the merits of various hypotheses and opinions. I, therefore, suggest that Drs. Richardson and Ellison be advised that the recommendations concerning their proposal stand unchanged and that a technical committee to investigate the question of reproductive isolation in screwworm populations will be established in the near future. The technical committee will be chaired by Dr. Leo LaChance, National Technical Advisor for Insect Genetics, and if possible, will include one of the scientists proposed in their letter.

Frankly, I am indignant over the attempt by Drs. Richardson and Ellison to discredit ARS scientists. The group that reviewed the proposal contained scientists of international stature who are certainly capable of evaluating research results and proposals concerning screwworms. Dr. LaChance is highly regarded internationally as an insect geneticist, has conducted research on screwworm genetics, and is the ARS National Technical Advisor for Insect Genetics. He did not, as stated, comment that he was not qualified to evaluate Drs. Richardson's and Ellison's findings. Dr. McInnis is a most competent insect geneticist in whom we have great confidence. Although he only spent 2 days at Austin, Dr. Ellison, himself, has spent several days at the Screwworm Research Laboratory in Mission working jointly

T. B. Kinney, Jr.

2

with Dr. McInnis on cytological techniques and reviewing data. The approximately 7 entomologists and 5 veterinarians who comprised the remainder of the group are all highly regarded scientists of impeccable integrity and scientific excellence. You will note from the enclosed list of participants that these individuals are the officials responsible for screwworm research and program implementation and are dedicated to the rapid, effective, and efficient execution of the program.



RALPH A. BRAM
National Research Program Leader
Insects Affecting Man & Animals

Enclosure

cc:

T. J. Army

H. G. Purchase

GENETIC DIFFERENCES AMONG SCREWORM POPULATIONS
REVIEW OF RESEARCH PLAN
Mission, Texas
November 10, 1981

Ralph A. Bram	USDA, ARS, Beltsville, MD
Edgar A. Taylor	USDA, ARS, Mission, TX
Wil Averhoff	Zoology Dept., U of TX, Austin
John Ellison	Zoology Dept., U of TX, Austin
Dick Richardson	Zoology Dept., U of TX, Austin
Dennis Ling	USDA, ARS, C/A, TX A&M, Mission, TX
Don McInnis	USDA, ARS, Mission, TX
R. C. Bushland	USDA Collaborator, McAllen, TX
R. A. Hoffman	USDA, ARS, College Station, TX
H. C. Hofmann	USDA, APHIS, Mexico City
C. J. Whitten	USDA, ARS, Fargo, ND
Floyd E. Smith	USDA, APHIS, Hyattsville, MD
Norvan Meyer	USDA, APHIS, Washington, DC
M. E. Meadows	USDA, APHIS, Mexico City
Moises Vargas Teran	Joint Com. Mexico City
Bud Turner	SWAHRF, NWG & NCA
Nazario Pineda	Joint Com. Mexico City
Leo LaChance	USDA, ARS, Fargo, ND
O. H. Graham	USDA, ARS, Mission, TX



United States
Department of
Agriculture

Agricultural
Research
Service

National
Program
Staff

Beltville, Maryland
20705

December 17,

SUBJECT: of Research at University of Texas

TO: T. E. Snney, Jr.
Administrator, ARS

THROUGH: T. E. Snney
Administrator, NPS

Purchase
Chief, LVS

ARS FACSIMILE MESSAGE

TO: Abraham PAGES: 2
FROM: Snney DATE: 12/17/61
JKT: NO: TIME: 12/17/61

In response to
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we contacted appropriate scientists who participated in
and have reevaluated all documentation associated with
which I was chairman. The review process was conducted in
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and on the best available scientific judgement. The partici-
pants, whom I have contacted, unanimously support my original

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T. H. Kinsley, Jr.

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program.

RALPH A. BRAM
National Research Program Leader
Insects Affecting Man & Animals

Enclosure

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United States
Department of
Agriculture

Agricultural
Research
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National
Program
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Beltsville, Maryland
20705

RECEIVED DEC 21 1981

Graham

December 17, 1981

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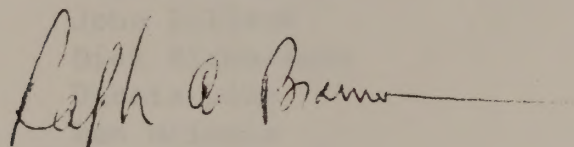
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Frankly, I am indignant over the attempt by Drs. Richardson and Ellison to discredit ARS scientists. The group that reviewed the proposal contained scientists of international stature who are certainly capable of evaluating research results and proposals concerning screwworms. Dr. LaChance is highly regarded internationally as an insect geneticist, has conducted research on screwworm genetics, and is the ARS National Technical Advisor for Insect Genetics. He did not, as stated, comment that he was not qualified to evaluate Drs. Richardson's and Ellison's findings. Dr. McInnis is a most competent insect geneticist in whom we have great confidence. Although he only spent 2 days at Austin, Dr. Ellison, himself, has spent several days at the Screwworm Research Laboratory in Mission working jointly

T. B. Kinney, Jr.

2

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A handwritten signature in cursive script, reading "Ralph A. Bram", followed by a horizontal line.

RALPH A. BRAM
National Research Program Leader
Insects Affecting Man & Animals

Enclosure

cc:

T. J. Army

H. G. Purchase

GENETIC DIFFERENCES AMONG SCREWORM POPULATIONS
REVIEW OF RESEARCH PLAN
Mission, Texas
November 10, 1981

Ralph A. Bram	USDA, ARS, Beltsville, MD
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Wil Averhoff	Zoology Dept., U of TX, Austin
John Ellison	Zoology Dept., U of TX, Austin
Dick Richardson	Zoology Dept., U of TX, Austin
Dennis Ling	USDA, ARS, C/A, TX A&M, Mission, TX
Don McInnis	USDA, ARS, Mission, TX
R. C. Bushland	USDA Collaborator, McAllen, TX
R. A. Hoffman	USDA, ARS, College Station, TX
H. C. Hofmann	USDA, APHIS, Mexico City
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Floyd E. Smith	USDA, APHIS, Hyattsville, MD
Norvan Meyer	USDA, APHIS, Washington, DC
M. E. Meadows	USDA, APHIS, Mexico City
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Leo LaChance	USDA, ARS, Fargo, ND
O. H. Graham	USDA, ARS, Mission, TX



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RECEIVED JAN 14 1982
Beltsville, Maryland
20705

January 8, 1982

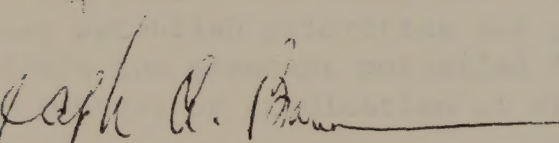
SUBJECT: Technical Committee for the Study of Potential Reproductive Isolation
in Screwworm populations

TO: ~~L. E. LaChance~~
MRRL, Fargo, North Dakota

Since the potential of reproductive isolation between screwworm populations in Mexico could be of critical importance to the further success of the screwworm eradication program, I wish to establish a technical committee of independent experts to study this question. I, therefore, request that you serve as chairman of such a committee in your capacity as National Technical Advisor for Insect Genetics.

As chairman of this technical committee, please prepare its terms of reference, develop all pertinent briefing materials, establish an itinerary and schedule of work, and appoint three prominent scientists of international stature to participate. When selecting scientists to serve on this technical committee, please consider including, if possible, one of the following who were recommended by Drs. R. H. Richardson and J. R. Ellison; Dr. Crodowaldo Pavan, Dr. M. J. D. White, Dr. Bruce Wallace, or Dr. Charles Michener.

I would recommend that arrangements be made with Dr. O. H. Graham to provide funding for this activity from resources of the Screwworm Research Project, Mission, Texas. Certainly, Dr. Graham and I are prepared to assist you in any way possible in order to assure the success of this most important assignment.


RALPH A. BRAM
National Research Program Leader
Insects Affecting Man & Animals

cc:

T. B. Kinney
T. J. Army
H. G. Purchase
E. L. Kendrick
P. J. Fitzgerald
J. R. Johnston
C. H. Schmidt
✓ O. H. Graham
C. J. Whitten
N. L. Meyer, APHIS

January 4, 1982

Dr. R. H. Richardson
The University of Texas at Austin
Department of Zoology
Austin, Texas 78712

Dr. J. R. Ellison
The University of Texas at Austin
Department of Zoology
Austin, Texas 78712

Dear Professors Richardson and Ellison:

This is a followup to my letter of December 14 regarding the review of the screwworm research that you have proposed. As I indicated in that letter, I asked Dr. Bram to reassess the review process and participants in order to make sure that he and other participants are satisfied that their recommendations are in order. A copy of Dr. Bram's response is attached.

I am satisfied that the review was adequate and the recommendations are proper. Thus, we cannot give further consideration to funding of the research you have proposed with the exception of the research on development of apodeme age and sterility determination. Dr. Bram will follow through on the establishment of a technical committee for the study of potential reproductive isolation in screwworm populations.

Gentlemen, we appreciated the opportunity to support some of your research in the past. You must, however, understand that with limited resources we must establish priorities and give support to that research that currently offers the greatest potential for contributing information that will lead to control or eradication of the screwworm.

Sincerely,

T. B. Kinney, Jr.

T. B. KINNEY, JR.
Acting Administrator

bcc: w/copy of incoming
Bud Turner

✓ R. Bram
N. Meyer, APHIS
A. Bertrand
H. G. Purchase
T. J. Army

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January 4, 1952

Dr. E. E. Schuchman
The University of Texas at Austin
Department of Zoology
Austin, Texas 78712

Dr. E. E. Schuchman
The University of Texas at Austin
Department of Zoology
Austin, Texas 78712

Dear Dr. Schuchman:

This is a letter to my friend, Dr. Schuchman, regarding the review of the manuscript of my paper, "The Biology of the Texas Culex tarsalis." I have been very interested in your review and the suggestions you have made. I am sure that your review will be very helpful in improving the manuscript. I am sure that your review will be very helpful in improving the manuscript.

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Sincerely,

T. B. Riney
T. B. Riney, Jr.
Austin, Texas

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SIX YEAR ACTION PLAN
FOR
THE APHIS SCREWORM PROGRAM

April 02, 1991

EXECUTIVE SUMMARY

This paper outlines the plan for fulfilling the USDA APHIS goal of preventing screwworms from re-entering the United States in the most efficient and cost-effective manner possible.

The plan includes three major interrelated components:

- 1) Establish a sustainable, stationary barrier in Panama.**
- 2) Retain the current production facility in Tuxtla Gutierrez, Chiapas, Mexico, as the sole source of sterile flies until a production facility is operating in Panama.**
- 3) Organize for an effective and efficient screwworm eradication program in Central America.**

GOAL

The goal of the USDA APHIS Screwworm Program is to prevent screwworms from re-entering the United States and other eradicated countries.

BACKGROUND The major mission of APHIS is the protection of the United States agricultural industry from foreign diseases and pests. Agriculture is the single largest industry in the United States and livestock production is the industry's largest component. Based on cash receipts, the livestock sector accounts for 55 percent of the total value of agriculture in the United States, not including the 40 percent of the plant sector dedicated to the production of animal feeds. An animal or animal product is the leading agricultural commodity in 34 of the 50 states.

The screwworm fly, *Cochliomyia hominivorax* (Coquerel), historically has been one of the most serious livestock pests, as well as a threat to humans, wildlife and other domestic animals. Adult flies lay eggs near open wounds; larvae that develop in these wounds cause serious injury and death if not treated.

Eradication of screwworms in the United States was one of the most significant animal health activities in the history of the livestock industry. It is estimated that screwworm eradication has saved producers, consumers and taxpayers hundreds of millions of dollars per year in terms of increased productivity and lower consumer prices. Eradication also has allowed significant changes to occur in livestock management and production; such as, a reduced need for labor in identifying and treating screwworm cases and an expansion of the livestock breeding season into spring and summer months when screwworm flies were most prevalent. Reintroduction and

establishment of screwworms would have a devastating effect on livestock production and marketing.

To reduce the risk of screwworms entering the United States from Mexico, APHIS initiated a program with Mexico in 1972 to create a barrier at the Isthmus of Tehuantepec. This highly successful program achieved that barrier in 1984; however, large movements of cattle from infested to eradicated areas proved to be an unacceptable risk. A moving eradication zone has progressed southward and currently is located in Belize and Guatemala. This moving barrier has accomplished the program goal of keeping screwworms out of the United States and Mexico.

This action plan is driven by the need to establish a sustainable barrier which can be maintained at minimal cost and provide an acceptable level of security to the United States and Mexico. At the same time, budget limitations and demands for greater accountability require a search for the most efficient and economical method to administer the program and to produce and disperse sterile flies.

In creating this plan, a number of assumptions were made about factors outside the program's control. These assumptions and more detailed economic analyses are contained in the Appendix. With these attendant uncertainties in mind, planning for the Screwworm Program takes on an even greater importance.

ISSUE 1. ESTABLISHING A BARRIER

ISSUE At present there is no permanent, sustainable screwworm barrier. The active eradication zone functions as a barrier as it moves south. Currently, the eradication zone has moved through Mexico and now lies within Guatemala and Belize. Selecting and establishing a stationary barrier at a strategically sound location will provide the most cost effective solution for protecting the livestock industry.

DISCUSSION In 1966 USDA declared the United States free of self-sustaining screwworm populations. At that time, it was considered sufficient to release sterile flies along the Mexico-United States border. The failure of this barrier in 1972 taught that the security a barrier provides is directly proportional to its distance from the United States and inversely proportional to its surface area. Shortly thereafter, APHIS signed an agreement with Mexico with the objective of eradicating screwworms to the narrowest part of Mexico, the Isthmus of Tehuantepec, and establishing a stationary barrier at that point.

When screwworms were eradicated to the Isthmus in 1984, it became obvious that a stationary barrier at this location would not be effective for two reasons. First, the large number of cattle produced in the Yucatan Peninsula and transported both legally and illegally into areas north of the Isthmus posed a high risk of continuously re-infesting screwworm-free areas. Second, the division created between livestock producers in the eradicated north and the infested south was politically unacceptable to Mexico.

Officials concluded that not only was a permanent barrier at the Isthmus of Tehuantepec costly and risky, but also it was not feasible to divide the country in half.

Sterile fly production accounts for over 50 percent of direct program costs; the smaller the land mass to be covered, the fewer flies need to be produced. Possible barrier or pause points included the Isthmus of Tehuantepec; the border between Guatemala, El Salvador and Honduras; Costa Rica; and the Darien Gap in Panama. An economic analysis in 1985 concluded that the most cost effective barrier would be located at the Darien Gap in Panama, the narrowest connection between the continents of North and South America. Panama was therefore selected as the most desirable site, with Costa Rica as an alternative location. Eradication activities have already moved into the Yucatan Peninsula, Guatemala and Belize in 1985, 1987 and 1989 respectively.

The major difficulty in completing eradication to Panama is a "hump" in dispersal levels of 262 million sterile flies per week for operations along the Honduras and Nicaragua border (see Table 1). When El Salvador and Honduras are eradicated, dispersal levels progressively decrease through Nicaragua and Costa Rica to 40 million sterile flies per week at Panama. The program conceivably can pause at any point but would require additional funding to sustain needed dispersal levels over larger geographical areas.

OBJECTIVE 1. By 1996 establish a permanent stationary barrier in Panama.

ACTION STEP	PRODUCT	RESPONSIBLE	DATE (q/yr)*
1. Negotiate agreements with: -El Salvador and Honduras -Nicaragua -Costa Rica -Panama --New Plant & Barrier --Eradication	Agreements with individual countries	DAIS **	2nd/91 4th/91 1st/92 *** 1st/94
2. Assess environmental impact	Environmental Impact Statement	DAIS	If and When Required
3. Discuss plan with USAID to obtain PL 480 funding for Central American Countries	Funding Commitment	DAIS/FAS	To be determined
4. Implement organizations for Central America and Panama	Infrastructures established	Regional Director	As scheduled
5. Conduct field operations (eradication, surveillance, quarantine, control, and support) to establish the barrier	Barrier established	Regional Director	1st/91 through 4th/96

* q = quarter, yr = year

** Deputy Administrator, International Services/APHIS

*** Informal communication with Panama indicates a willingness to accept both a production facility and a permanent barrier.

ISSUE 2. INFRASTRUCTURE FOR PRODUCING STERILE FLIES

ISSUE The screwworm production facility located in Tuxtla Gutierrez, Chiapas, Mexico, is the only functioning plant of its kind in the world. Deficiencies in the plant's physical condition have reached the point where a significant, continued commitment of funds is required to ensure reliable production of sterile flies to reach and establish a barrier in Panama. Renovations are also required to incorporate technological changes in rearing methods developed since the plant was built in 1973. Probable budgets through Fiscal Year 1996 do not include sufficient funds to meet all these needs at one time.

Long-term sustained production for barrier maintenance in Panama requires a new facility located close to the barrier. The size of such a facility would be appropriate for the required dispersal of 40 million sterile flies per week. The facility at Tuxtla Gutierrez, designed to produce 500 million sterile flies per week, cannot be operated in a cost-effective manner at 8% of capacity.

DISCUSSION Reliable sterile fly production is necessary to safeguard progress to date and to advance into Central America. Deficiencies in the Tuxtla Gutierrez rearing facility include a wide variety of significant cost items.

The total cost for bringing the facility up to standard is approximately \$13 million. A contribution from FAO of \$1.6 million for renovation will be used to support the eradication effort in North Africa.

The program strategy of establishing the barrier in Panama, combined with budget limitations, requires that repairs and replacements are systematically and consistently carried out. The current best estimate is that approximately \$3.0 million is required annually. These funds will be needed regardless of the eradication zone's location and for as long as the Tuxtla Gutierrez plant is in operation.

Upon reaching Panama, the most cost effective method of providing flies for barrier maintenance is through a new rearing facility located close to the barrier. Although extensive design for a facility in Panama was completed in 1987, the design criteria and cost estimates must be revised to reflect current program planning and new technologies. Since additional USDA funds probably will not be available for a new plant, alternative sources of funding and organizational relationships should be explored including leasing arrangements or other private market solutions. Discussions toward the identification of private contract and funding sources must be initiated soon.

CURRENT STATUS A new supply water treatment plant is in operation which should significantly reduce further wear on water conducting components of the Heating Ventilation and Air Conditioning (HVAC) system. The water distribution system is being reviewed and repaired to reduce water loss. The next step will be to address mechanical deficiencies of the HVAC system. The facility must be able to meet a field need of 262 million insects per week by the first quarter of 1992.

ISSUE 3. ORGANIZING FOR EFFECTIVE AND EFFICIENT SCREWORM ERADICATION IN CENTRAL AMERICA

ISSUE Future APHIS screwworm eradication activities in Central America will be cooperative efforts between the United States and each cooperating country with sterile flies being provided by the Mexico-United States Commission for the Eradication of Screwworms. This creates the need to restructure the Commission; organize efficient and effective program operations in each host country; and, ultimately, barrier maintenance operations in Panama.

DISCUSSION Since the inception of screwworm eradication efforts in Mexico in 1972, activities in that country have been bilaterally managed through the Mexico-United States Commission. The nature of this Commission will change as APHIS moves into Central America. The Commission will provide sterile flies for the USDA eradication effort in Central America. Mexico will assume prevention and surveillance responsibilities once screwworms become an exotic pest in that country.

One of the critical challenges facing APHIS is the need to design and implement an organizational structure to best meet program needs in the new operational environments. The Central American countries are geographically small,

their national budgets lack the resources to finance the program directly and the eradication efforts in any one country are not expected to last more than two to three years. These factors require a well coordinated APHIS program and host country structures that can best accommodate the varying operational conditions on a country-by-country basis.

Once screwworm eradication is accomplished, the remaining host country's infrastructure must conduct prevention and surveillance activities and future animal and plant health activities.

In order to establish a permanent barrier in Panama, a new rearing facility will need to be brought on line for three reasons. First, maintaining a rearing facility in the eradicated zone will present an unacceptable risk due to the presence of fertile material; second, field operational costs would be significantly higher utilizing the existing facility due to the need to transport sterile pupae to Panama; and third, operational costs would be significantly higher operating the existing facility at only 8% of capacity. APHIS will need to work with Mexico and USAID to find an alternate use for the old facility and alternate employment opportunities.

APPENDIX

APPENDIX

Economic and Production Considerations

I. Introduction

This section contains a more detailed discussion of the production and economic calculations and assumptions of the APHIS Screwworm Plan.

II. Program Model

A. Barrier Location

The goal of the Screwworm Program is to prevent screwworms from re-entering the United States and other eradicated countries. Selection of a sterile fly barrier location is based on two criteria: (1) the relative degree of risk/security the location provides; and, (2) the cost of maintaining the barrier. The more distant the barrier, the lower the risk of screwworms re-entering an eradicated country. Total program cost is a function of the land surface area the barrier must cover, sterile fly transportation and dispersal, labor, and materials. Generally, surface area is the most important factor determining total barrier cost.

The program's original goal was to establish and maintain a sterile fly barrier at the Isthmus of Tehuantepec, approximately 200 miles in width. The introduction of new technology, such as a gelatin based diet and a box-free adult dispersal system, will enable the barrier to move to a more cost effective location further south.

A barrier at the Isthmus of Panama (southeast of Panama City, near the Darien Gap) maximizes the security of the

United States against screwworm re-infestation and minimizes the long-term costs of maintaining the barrier and the program as a whole. A 1985 study of the "barrier location problem" concluded Panama is clearly the optimal location among the available sites in Central America. Costa Rica is the second most favorable location for a barrier and a future sterile fly production facility; however, Costa Rica's mountain ranges add a significant element of risk to the task of aerially releasing flies to maintain the barrier. The size of the barrier needed in Costa Rica would require 60 million sterile flies per week, fifty percent more than in Panama (40 million sterile flies per week). As the eradication effort proceeds south, it provides a moving barrier that could be maintained at any given location if the program had to stop for any reason. Clearly, however, maintaining a barrier north of Panama is logistically, financially and politically more difficult to sustain.

B. Program Model

Table 1 shows a sterile fly dispersal plan for the Central American countries which would, given certain assumptions, eradicate screwworms from the area by 1996. The plan provides for a gradual buildup of sterile fly releases over a six-month period for each country with the exception of El Salvador, one calendar year of full dispersion (3,000 sterile flies weekly per square mile), and then a six-month wind-down period. Sterile flies will be flown from the current facility in Tuxtla Gutierrez, Chiapas, Mexico, to the active eradication area. Table 1 shows that the number of sterile flies needed to implement this plan peaks at approximately 262 million insects per week in the first quarter of the second year (the first quarter of 1992 if the program proceeds on schedule).

C. Budget Analysis

Table 2 provides a detailed breakdown of the anticipated costs for one year of an eradication program in each of the Central American countries. Overhead costs are not included. A key factor is the distance of each country from Tuxtla Gutierrez and the anticipated pupae transport and air dispersal costs. Twin engine aircraft are required to disperse sterile flies throughout Central America (with the possible exception of Panama, an additional costs advantage). Such aircraft cost twice as much per hour as the single engine aircraft previously used by the Commission. Table 3 provides a breakdown of the anticipated costs of the program through 1996.

Table 4 presents the costs of maintaining a barrier at the Isthmus of Tehuantepec. Maintaining a barrier at this location would entail significantly higher costs compared to Panama. A barrier at Tehuantepec cannot be sustained through 1996 with current funding; a budget increase of approximately \$7 million annually (30% more than current spending) would be needed to maintain a barrier that would provide significantly less security to the United States livestock industry and its consumers than a barrier anywhere else in Central America.

III. Assumptions

A. The Program's Real Dollar Budget Will Decline

The real dollar budget (i.e., the net funds available to the field, corrected for inflation) will decrease. This will occur as the inflation rate in the Central American countries (estimated at 6% after factoring in changes in exchange rates) outstrips the assumed 3 percent annual inflationary

increase in funds available to the field. Given the U.S. government's current fiscal condition, no additional increases in program funding can be expected.

B. Appropriated Funds For A New Production Plant Will not be Available

No money is expected to be available from appropriations in the immediate future to build a new sterile fly rearing plant. Furthermore, it was assumed that no funds will be available from other sources to support the program directly.

C. The Current Facility Will Produce Sufficient Sterile Flies To Support the Program

The program may be continued using pupae transported by air from the Tuxtla Gutierrez plant. Although this has some risks such as the risk of having the plant in a screwworm-free area, lack of infrastructure in the eradicated countries and costly long-distance air transport, it is clearly feasible, both technically and economically. A new facility will be required in Panama eventually.

D. Returning the Barrier to the Isthmus of Tehuantepec is the Least Preferred Alternative

By the end of Fiscal Year 1991, the program will be releasing sterile flies over all of Guatemala and Belize. Returning the barrier to the Isthmus of Tehuantepec would sacrifice the gains made since 1986. It would also be impossible to sustain the program at current funding because returning to Tehuantepec would require rebuilding the extensive and expensive field staff for surveillance in Mexico. A preferred alternative would be to create an interim barrier in Honduras and El Salvador.

E. Agreements are Signed on Schedule and No Other Significant Delays Occur

The program model assumes that agreements with the Central American countries will be signed on schedule. Delays in obtaining these agreements will lengthen the program and increase costs. Delays caused for other reasons (e.g., any interruption of sterile fly production) would have the same impact.

F. The Program Will Be Led By APHIS

To achieve eradication in the area by 1996 it is essential that APHIS operate the program cooperatively with the various host country governments. As noted in the Plan, the Mexico-United States Commission will continue to provide sterile flies for the program.

G. Administrative Cost Savings Will Be Achieved

Program funding is assumed to decline in real dollars (e.g, "constant dollars", or face value of funding corrected for inflation to show buying power in 1990 dollars) available to the field. It will be necessary to make significant savings in areas such as purchasing and personnel if anticipated funding is to provide adequate resources for a Central American program. Without such savings, the funds available in the first 2 years will be insufficient to run the program as planned (Table 3). The Planning Team believes that these savings can be achieved.

H. Significant Outbreaks are Paid Through Emergency Funds

The anticipated budget outlined in Table 3 contains no contingency funds to eradicate large outbreaks in screwworm-free areas. These funds would have to be provided from other sources to keep the program operating within its budget and on schedule.

I. This Plan is Initiated During Fiscal Year 1991

It is essential that this entire program be initiated without delay and be carried out according to the recommended schedule. If major delays are encountered or unexpected budget reductions occur, the final goal will not be met.

TABLES AND FIGURES

Table 1. Sterile fly dispersal needs by country for eradicating screwworm
from Central America

Year and Quarter																								
Country	1991				1992				1993				1994				1995				1996			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
millions of flies per week																								
Mexico	40	40	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Guatemala	115	115	115	115	90	60	30																	
Belize	24	24	24	24	18	12	6																	
Honduras			30	60	120	120	120	120	60	30														
El Salvador			24	24	24	24	24	24	12	6														
Nicaragua							30	60	125	125	125	125	60	30										
Costa Rica											15	30	60	60	60	60	30	15						
Panama															20	40	80	80	80	80	40	40	40	40
Total	179	179	203	233	262	226	220	214	207	171	150	165	130	100	90	110	120	105	90	90	50	50	50	50

Table 2. Projected screwworm program cost by country 1/

Cost Item	Country						
	Belize	Guatemala	Honduras	El Salvador	Nicaragua	Costa Rica	Panama
	----- Millions of 1991 U.S. dollars -----						
Fly Diet	0.5	2.5	2.6	0.5	2.7	1.3	1.7
Pupae Transport 2/	0.2	0.8	0.9	0.2	1.2	0.7	1.1
Air Dispersal	0.7	3.5	3.7	0.7	3.8	1.8	2.5
Boxes	0.2	0.9	0.9	0.2	0.9	0.4	0.6
Packaging 3/	0.1	0.3	0.3	0.1	0.3	0.1	0.2
Field Operations	0.5	1.5	1.5	0.5	2.0	1.0	1.5
Total	2.2	9.4	9.9	2.2	11.0	5.4	7.5

1/ Cost in constant dollars (1991 US\$) for direct program costs for each country for one year.

2/ Based on air transport of pupae from Tuxtla Gutierrez, Mexico, to a packaging center in each country. Number of pupae shipped weekly during peak dispersal periods and air miles from Tuxtla Gutierrez to packaging centers as follows: Belize--24 million pupae/week, 281 miles; Guatemala--115 million pupae/week, 281 miles; Honduras--120 million pupae/week, 410 miles; El Salvador--24 million pupae/week, 305 miles; Nicaragua--125 million pupae/week, 491 miles; Costa Rica--60 million pupae/week, 678 miles; Panama--80 million pupae/week, 1,004 miles.

3/ Cost of operating packaging centers to box bulk pupae for air dispersal.

Table 3. Projected funding, expenses and surplus/shortfall by year for Screwworm Eradication Program in Central America 1/

	1991	1992	1993	1994	1995	1996	Total
----- millions of 1991 U.S. dollars -----							
SOURCES OF FUNDS							
Mexico	3.5	3.6	3.7	3.8	3.9	4.1	22.6
United States	28.1	29.2	30.1	31.0	31.9	32.9	183.6
Total	31.6	32.8	33.8	34.8	35.9	37.0	206.2
EXPENSES							
80/20 Budget							
Administration	1.3	1.0	0.5	0.0	0.0	0.0	2.8
Field Operations	3.3	0.0	0.0	0.0	0.0	0.0	3.3
Production	8.5	9.0	9.6	10.1	10.7	11.4	59.3
Air Operations	0.1	0.0	0.0	0.0	0.0	0.0	0.1
Subtotal	13.2	10.0	10.1	10.1	10.7	11.4	65.5
Country Programs							
Mexico	0.9	0.2	0.2	0.3	0.3	0.3	2.2
Belize	2.2	0.9	0.0	0.0	0.0	0.0	3.1
Guatemala	9.4	3.8	0.0	0.0	0.0	0.0	13.2
El Salvador	1.1	2.3	0.5	0.0	0.0	0.0	3.9
Honduras	1.9	10.5	2.1	0.0	0.0	0.0	14.4
Nicaragua	0.0	2.2	12.3	2.4	0.0	0.0	16.9
Costa Rica	0.0	0.0	1.1	6.4	1.3	0.0	8.9
Panama	0.0	0.0	0.0	1.7	9.5	5.0	16.2
Subtotal	15.5	19.8	16.2	10.8	11.1	5.3	78.8
Support Budget							
U.S. Support	5.2	5.6	5.9	6.2	6.6	3.5	33.0
Plant Maintenance	0.5	0.5	0.5	0.5	0.5	0.5	3.0
Air Supervision	0.1	0.1	0.1	0.1	0.1	0.0	0.4
Mission Plant	0.2	0.2	0.2	0.2	0.2	0.2	1.1
Subtotal	6.0	6.3	6.6	7.0	7.4	4.3	37.6
GRAND TOTAL	34.6	36.1	32.9	28.0	29.2	21.0	181.8
YEAR-END BALANCE	(3.1)	(3.3)	0.9	6.9	6.7	16.0	24.4

1/ Eradication schedule as shown in Appendix Tables 1 and 2; permanent stationary barrier established in Panama in 1986. Figures rounded to nearest 100,000. Projections reflect a 3% yearly increase due to inflation.

Table 4. Projected funding, expenses, and surplus/shortfall by year for establishing and maintaining sterile-fly barrier at the Isthmus of Tehuantepec, Mexico 1/

	1991	1992	1993	1994	1995	1996	Total
----- millions of 1991 U.S. dollars -----							
SOURCES OF FUNDS							
Mexico	3.5	3.6	3.7	3.8	3.9	4.1	22.6
United States	28.1	29.2	30.1	31.0	31.9	32.9	183.6
Total	31.6	32.8	33.8	34.8	35.9	37.0	206.2
EXPENSES							
80/20 Budget							
Administration	1.5	1.6	1.7	1.8	1.9	2.0	10.5
Field Operations	10.0	10.6	11.3	12.0	12.7	13.4	70.1
Production	11.8	12.5	13.2	14.0	14.9	15.8	82.1
Air Operations	4.5	4.8	5.1	5.4	5.7	6.0	31.4
Subtotal	27.8	29.5	31.3	33.1	35.1	37.2	194.1
Support Budget							
U.S. Support	5.2	5.6	5.9	6.2	6.6	7.0	36.6
Plant Maintenance	0.5	0.5	0.5	0.5	0.5	0.5	3.0
Air Supervision	0.1	0.1	0.1	0.1	0.1	0.1	0.4
Mission Plant	0.2	0.2	0.2	0.2	0.2	0.2	1.1
Subtotal	6.0	6.3	6.6	7.0	7.4	7.8	41.1
GRAND TOTAL	33.8	35.8	37.9	40.1	42.5	45.0	235.2
YEAR-END BALANCE	(2.1)	(2.9)	(4.1)	(5.3)	(6.6)	(8.1)	(28.9)

1/ Re-establishing a permanent sterile fly barrier at the Isthmus of Tehuantepec and maintaining it there indefinitely. Funding assumes 3% annual increase for inflation. Figures are rounded to nearest 100,000.

SCREWORM ERADICATION IN CENTRAL AMERICA

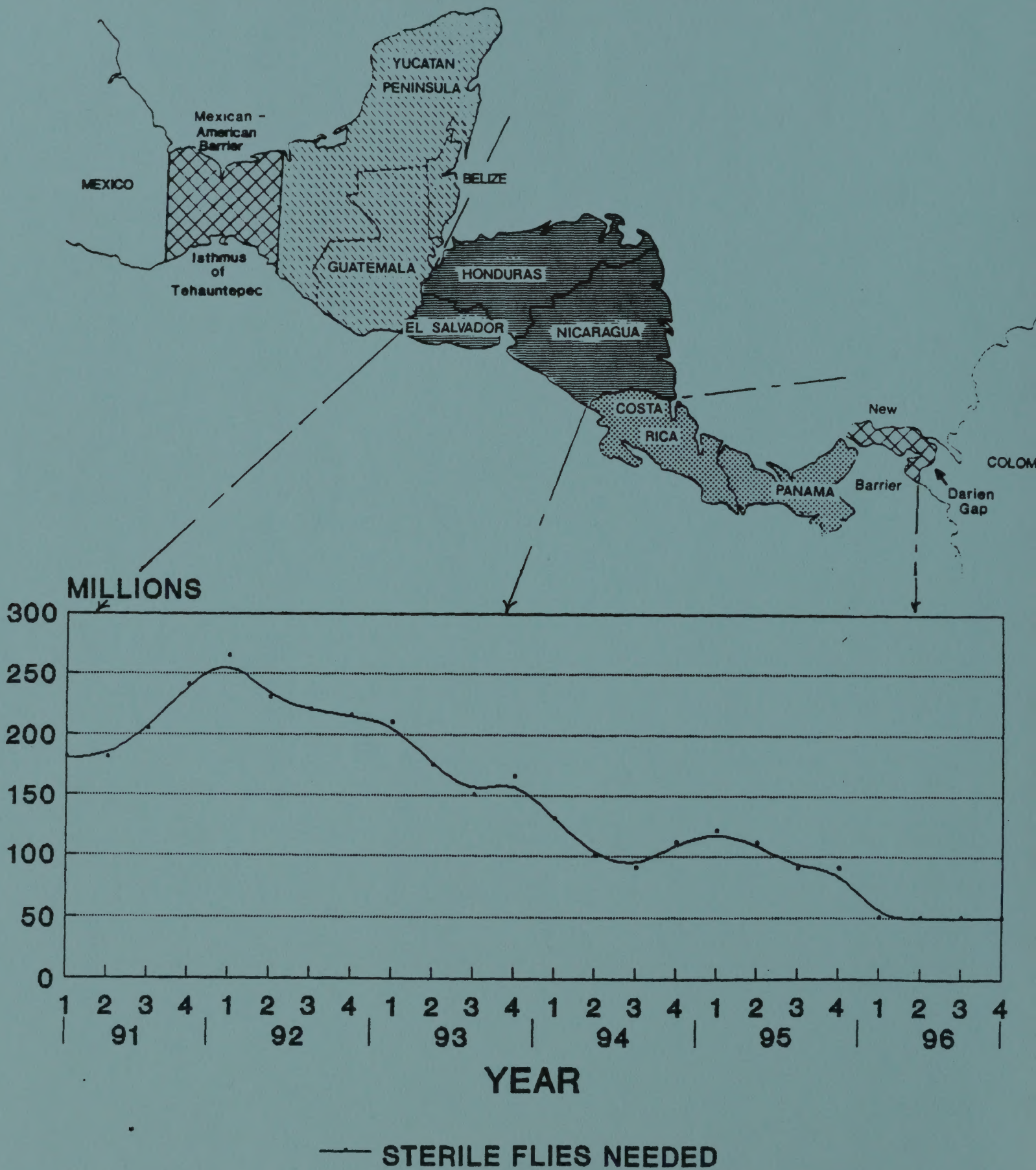
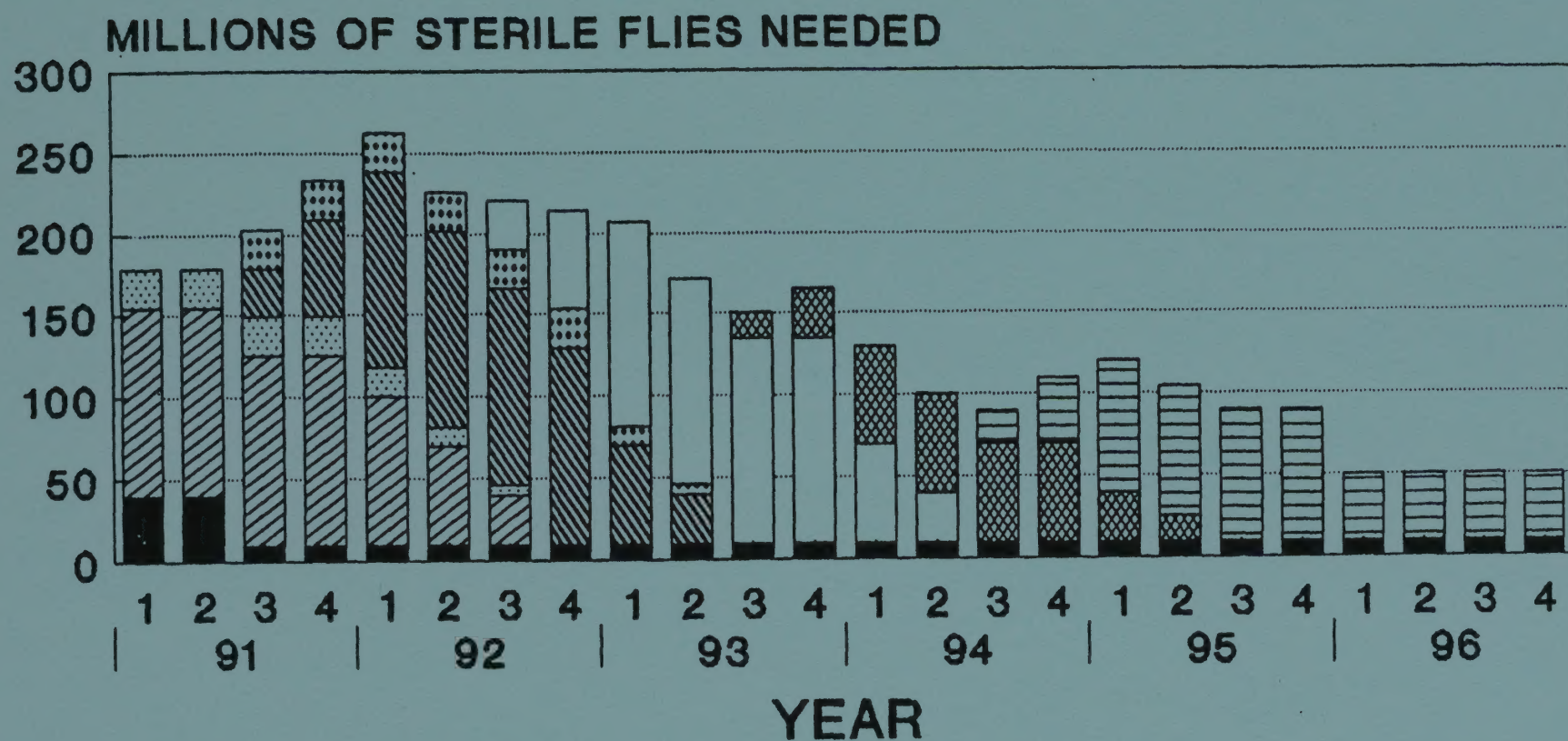


Fig. 1. Relationship between eradication zones and the number of sterile insects required to reach pause/barrier points.

SCREWWORM ERADICATION

CENTRAL AMERICA



■ MEXICO ▨ GUATEMALA ▤ BELIZE ▩ HONDURAS
 ▧ EL SALVADOR □ NICARAGUA ▦ COSTA RICA ▨ PANAMA

GENETIC DIFFERENCES AMONG
SCREWORM POPULATIONS

USDA Cooperative Agreement 58-7B30-9-120

Annual Report

November 4, 1981

R. H. Richardson, J. R. Ellison & W. W. Averhof



THE UNIVERSITY OF TEXAS AT AUSTIN
THE GENETICS INSTITUTE
AUSTIN

OHG



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ANNUAL REPORT: COOPERATIVE AGREEMENT 58-7B30-9-120

GENETIC VARIATION IN SCREWORMS

University of Texas at Austin

R. H. Richardson, John R. Ellison, W. W. Aaverhoff

1.0 WHERE WE ARE

In 1978 we reported extensive genetic diversity of non-interbreeding populations (gamodemes) of screwworms in the U.S. Later surveys into Mexico identified some of the overwintering areas of these infestations. Further sampling has continued to uncover gamodemes, with a maximum of seven in the most southern area of Chiapas. The diversity in Mexico has been complex and extensive. Thus far the sampling has been superficial for most areas. Although the evolutionary significance of this diversity has been interesting from a basic science point of view, the behavioral, phylogenetic and ecological aspects of this diversity have been largely untouched. The major focus of our work has been to relate this diversity to the effectiveness of the screwworm eradication program in Mexico. Until the Arriaga Test (a cooperative venture between ARS and U. of Texas) we had circumstantial evidence that this diversity was sometimes important, but alternative explanations were also prevalent. The Arriaga Test, including the following test of the V-81 strain, has now given us direct evidence that the diversity is important to the program. Furthermore, the result of this test demonstrated the validity of our earlier hypothesis regarding the roles that genetic diversity may have played in previous control experiences.

Results of many biogeographic studies of plants and animals across many taxonomic classes repeatedly have shown increasing genetic (taxonomic) diversity as samples are taken nearer to centers of phylogenetic origin and/or from temperate to tropical zones. Since screwworms are presumed to be of tropical origin, both possibilities lead us to expect to encounter an increased diversity southward. Results to date are consistent with this expectation.

As the diversity of screwworms at more southern locations in Mexico (and presumably in Central America) increases, the problems of producing effective sterile flies are complicated. Although successful eradication may appear to have been achieved in some areas at the time, the perfection even in northern areas may be an illusion. Using the present procedures outbreaks north of the release zone may be expected to continue because of incomplete eradication, leaving the U.S. chronically at risk to a serious outbreak in years favorable for screwworms. This situation may be corrected if the types of screwworms causing the outbreaks can be identified, and eliminated by an appropriate type of sterile fly. (Of course, re-introductions are no less a problem.)

Complete eradication may be expected by using several strains matching the population under treatment, or, probably, a "fruit cocktail" mixture of strains concocted without forced crossing. An effective mixture for release might be formed from types that were egged separately but grown together in the vats in the factory. Assuming no more than 5 types of screwworms in most areas of treatment, a release rate of about 500 flies/square mile should be adequate except for very unusual situations. But, it is essential that a thorough survey of the screwworm diversity be made before release begins in an area, and continual genetic monitoring of the cases in the release area would be necessary to detect the appearance of previously unfound types.

The presence of an asexual screwworm case we saw this summer adds an additional dimension of genetic diversity to consider. While there may be a low heritability for the trait, it would almost certainly be at a selective advantage when sterile flies are being released. Should a parthenogenetic population become established, its control would not be possible by release of sterile flies.

2.0 THE ARRIAGA TEST

This extensive field test was a joint venture between USDA/ARS research staff and our group. Collections were made in 1980 to gather baseline information on the diversity in the proposed test area and to obtain cultures from which to select material for producing sterile flies. These collections were made from April 29th to July 30th, producing 1269 egg mass samples, with 161 completed karyotypic analyses.

2.1 Background

1. The first test consisted of two strains, the ARS "broad based" strain, V-81, and the UT "narrow based" strain, RGM-6. V-81 was formed by forced crosses of several collections, most of which were Type-F, but with a small number of Types -A and -B. RGM-6 was derived from a single egg mass (one pair of wild flies) of Type-F.
2. The test area was divided into three plots, one on each end receiving either sterile flies from V-81 (area C) or RGM-6 (area A). The area in the middle (area B) did not have flies released in it, but the results suggest that there were extensive movements of sterile flies into area B from both area A and area C.
3. V-81 and RGM-6 were released on 1-mile swaths (2-mile flight lanes that were interdigitated in alternate releases). The release rate for the first 4 weeks was 125 flies/square mile, followed by a 10 day gap with no releases, and then 320 V-81

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flies/square mile and 220 RGM-6 flies/square mile were released during the last 3 weeks.

4. There was a period of 3 weeks during which the area received no flies, but egg mass sampling was continued.
5. The strain test using V-81 has been in progress for about 2.5 months at a release rate of 1500 flies/square mile. Most of the sterility results are in hand.

2.2 Questions And Rationale

Several questions were asked in the field test. The primary objective was to determine the extent of sexual isolation among types of screwworms in the area. The test was initiated with a release lower than any previously used in the eradication of screwworms using two sources of sterile flies. RGM-6 was considered a "narrow base" since it was derived from two individuals. V-81 was a "broad base" derived from many individuals including three gamodemes. Fortuitously, V-81 and RGM-6 were representatives of much the same gene pool, Type-F, since V-81 had only a minor component of types A and B.

Secondary objectives included (1) measuring any differences in strength of isolation between native and released flies; (2) comparing effectiveness of a single egg mass strain to a multiple egg mass strain; (3) monitoring changes in relative frequencies of any types not eliminated from the area to give a base line for any changes which may occur over a longer time and be detected in the future; (4) comparing factory productivity of a single egg mass strain with that of a broad base strain; (5) projecting the implications of the extent of sexual isolation and its effects on the eradication program.

Measuring the differences in strength of isolation between native and released flies was based on the supposition that low release rates of sterile flies would affect only those with little or no isolation, and increasing the release rate would overwhelm progressively more strongly isolated types. Since V-81 was similar to RGM-6 in genetic composition, the following strain test allowed this objective to continue into this aspect of the studies in this area, although it was not a part of the cooperative studies between USDA/ARS and UT.

2.3 Results Of The First Test (Low Release Rates)

The screwworm population in the area was comprised of 7 types of screwworms -- A, B, C, D, E, F and I. The results are graphed in Figure 1. The reference points are indicated for frequencies of the types found in 1980 and before the test in 1981, followed by frequencies obtained as

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a result of the field test. Except for the absence of Type-I, these frequencies are within sampling error of this area during the summer and fall of 1980. Weighted averages are shown. Type-F was the most common, with an initial frequency of about 39%, followed by Type-E (20%), Type-D (16%), Type-A (11%), Type B (9%), Type-C (3%), and Type I (2%), and Type-C (2%). A similar pattern was seen in area C, where V-81 was released, as in area A. By the end of the test in areas A and C, Type-F was absent, Type-E declined about one third to 13%, Type-D had tripled to become the most common (53%), Type-I had increased to 20%, and the rest had not changed significantly. At this stage of incomplete analysis of the collections there is no significant difference between the effectiveness of RGM-6 and V-81.

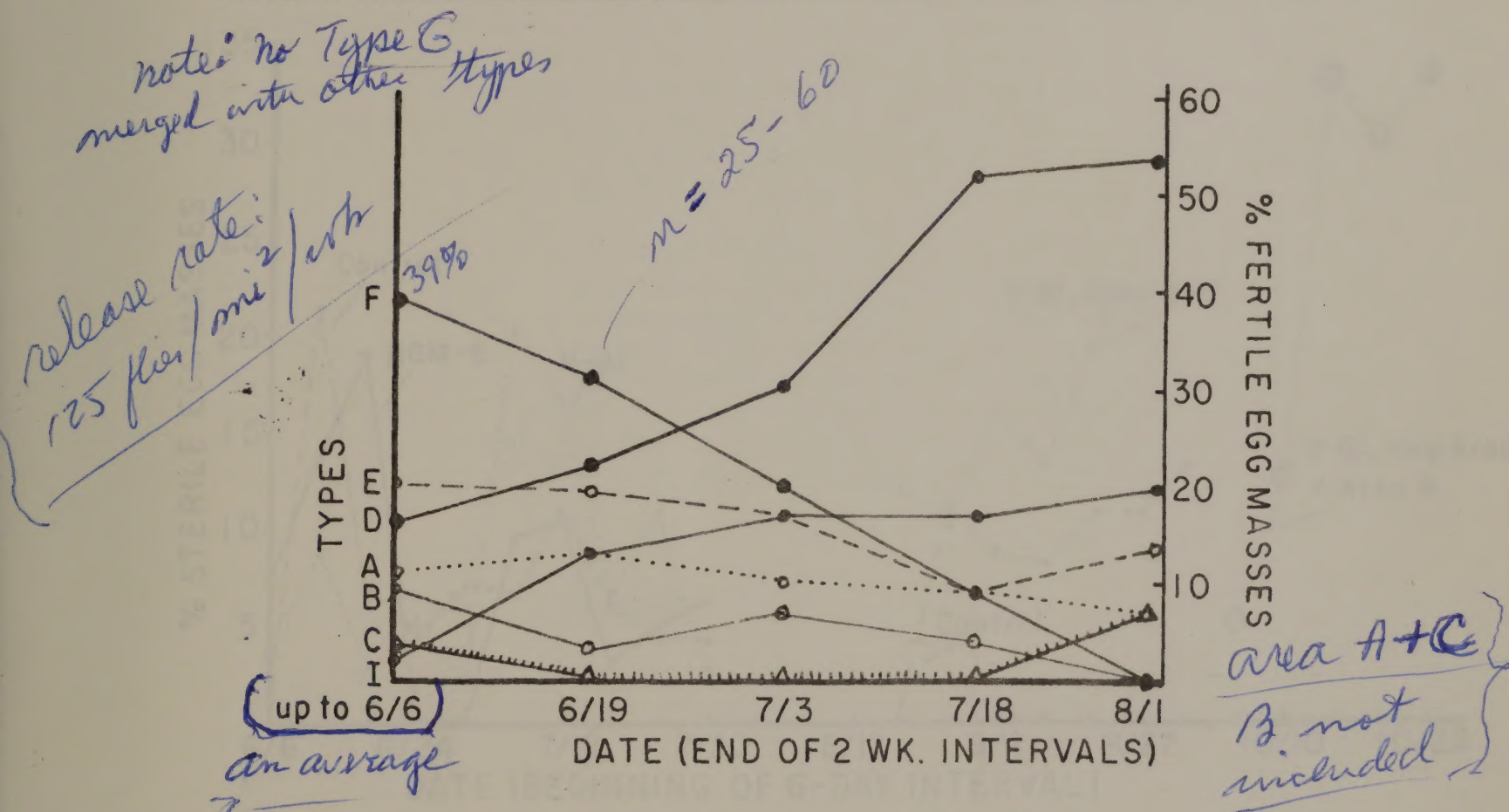


FIGURE 1. Relative frequencies of types of screwworms during release of low numbers of sterile flies from RGM-6 and V-81 lines, Arriaga, Chiapas. *(based on 161 of 1269 egg masses)*

Gamodemes are indicated at left margin of graph, and reported on bi-weekly intervals except for the initial points. The initial frequencies are the weighted average for the frequencies in 1980, and from pretest collections in May and first week of June, 1981.

The sterility during this test was low overall (Figure 2). However, the results shown in Figure 1 clearly show the sterile flies were having a profound effect on the native flies. The reproduction of Type-F was

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stopped abruptly, and there were shifts in the remaining types. The increase of Type-D was spectacular, and the increase in Type-I was significant. Instead of all residual types increasing proportionally to the elimination of Type-F, there were subtle shifts or no shifts in most types, but Type-D increased to become the dominant type, while Type-I comprised the bulk of the remaining population. This is known among population biologists as a "species release" where the removal of a limiting feature in the reproductive processes of a population causes it to undergo a dramatic increase in growth rate. While we have no way of identifying the limiting factors, we presume that competition between Type-F and Types-D and -I were a major component. This is the type of response that we postulated had happened repeatedly in the past, where

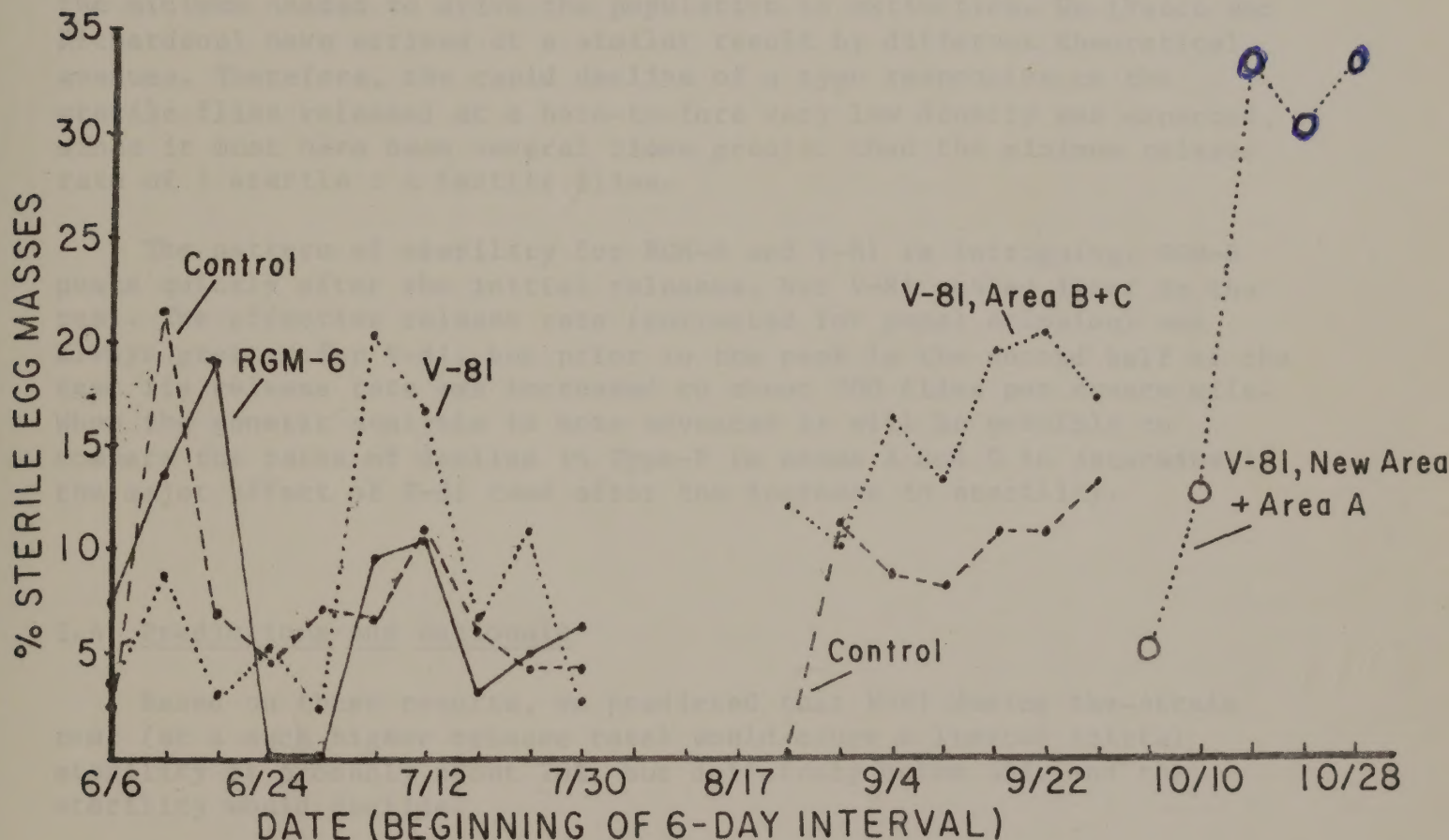


FIGURE 2. Proportion of egg masses failing to hatch

Collections were made from wounded sheep daily, and averages for 6 consecutive days plotted. (This time interval is to be comparable to the time scale in Figure 3.) Releases of sterile flies from 6/6 to 6/28 were about 125 per square mile per week; from 7/8 to 8/4 about 250-300 sterile flies per square mile per week were released. No sterile flies were again released until 8/23, when the test of the V-81 strain test was initiated. Release rate was about 1500 sterile flies per square mile per week. From 8/23 to 10/3 sterile flies were released in the areas originally designated B and C. Beginning on about 10/4 release of sterile flies was shifted to the original area A and included some areas outside this area, not previously treated.

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sterile flies similar to a dominant type effectively drove this type to extinction, and subsequently another type, sexually isolated from the sterile flies, increased to become a dominant type. A new collection for the factory production would begin the cycle again, since it would be comprised mostly of the dominant type. This procedure would repeat until all the types in an area were obliterated.

These results also help to verify the general validity of theory based on density dependent models for limitations of population size. For example, Barclay and Mackauer (Envt. Ent. 9: 810. 1980.) have shown such a model predicts that the release of sterile flies equal to about 25% of the carrying capacity (natural population equilibrium density) is the minimum needed to drive the population to extinction. We (Vasco and Richardson) have arrived at a similar result by different theoretical avenues. Therefore, the rapid decline of a type responsive to the sterile flies released at a here-to-fore very low density was expected, since it must have been several times greater than the minimum release rate of 1 sterile : 4 fertile flies.

The pattern of sterility for RGM-6 and V-81 is intriguing. RGM-6 peaks quickly after the initial releases, but V-81 peaked later in the test. The effective release rate (corrected for pupal eclosion) was always greater for V-81, but prior to the peak in the second half of the test its release rate was increased to about 300 flies per square mile. When the genetic analysis is more advanced it will be possible to compare the rates of decline in Type-F in areas A and C to determine if the major effect of V-81 came after the increase in sterility.

2.4 Predictions And Rationale

Based on these results, we predicted that V-81 during the strain test (at a much higher release rate) would cause a limited initial sterility of probably about 20%, but definitely below 50%, and the sterility would decline.

The rationale was based on the frequency of Type-F having been reduced to a low level during the previous testing at low release rates, and Types-D and -I having increased in proportion during the release. Knowing that Types-D and -I were highly resistant to the low level release (D perhaps more so than Type-I), the prediction was made that they would also be resistant to releases at higher levels. These two gamodemes accounted for more than 65% of the total fertile egg masses during the last 4 weeks of the Arriaga test. The initial sterility should be comparable to the sterility in the earlier tests, but with some unknown increase due to a higher release rate affecting other lines. As Type-F was again reduced to essentially zero in the population, we would expect the sterility to decline. If any other types were eventually driven out, the sterility would decline further since only the types having sufficient isolation to retain their ability to reproduce effectively in the presence of sterile flies would be present.

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Thereby our rationale was essentially a restatement of the effects just observed, with an additional effect added for the increased release rate affecting some types with intermediate strengths of isolation. We did not expect Type-D to be overwhelmed since it had shown such a marked expansion in the earlier test.

2.5 Results Of The V-81 Strain Test

For the first part of this test, the sterility peaked at about 20% (Figure 2) on 6-day intervals (ca. 25% on 7-day intervals, now shown). We have not confirmed the removal of Type-F, and possibly others which may have been affected, but the presence of some types (such as Type-D and Type-I) not responding to V-81 are apparent from the fertile egg masses still being obtained.

The first portion of this curve (8/23-10/3) was with a release of sterile flies in areas B and C, which had been previously treated (area B by dispersal, area C by air drop). The percent sterility increased slightly. The major change came in the period after 10/4, when the area treated was moved to area A and beyond any area previously treated. Area A had not received any direct treatment for two months. Even here the sterility was up to only 30-35%, not far from that expected if the primary effect is on Type-F, which likely had recovered in this area, not to mention the prospects for migration down the coast. Subsequent genetic analyses should clarify shifts in this new area.

The total population has remained remarkably constant during the entire field test, regardless of release rates used (Figure 3). Although the measure, number of egg masses per 6-day period, is crude for relative numbers of fertile females, it is useful if certain extraneous factors are considered. For example, when newly wounded animals are added (as often is done when a new collection pen of sheep is established), a delay of 2-4 days is experienced before the attractiveness of the pen stabilizes. A sizeable proportion of new pens and wounded animals reduces the collections enough to noticeably affect an average of several days. Also collections are always sparse when the weather is continuously rainy. The abrupt decline between August 23 and August 29 (circled point) reflects a combination of these two effects. Otherwise, the pattern of fluctuations seem largely random effects (or at least haphazard).

2.6 Culturability Of Broad Based And Narrow Based Strains

The evaluation of this comparison was primarily the responsibility of ARS staff. The initial feelings were that RGM-6 was more sensitive to environmental perturbations, and that it had a lower eclosion percentage. During the time when sterile flies were being released there were serious problems with temperature control, and Dr. Martinez

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(Mexico-American Commission) indicated that he considered the differences seen were explainable by the differences seen in culture conditions to which V-81 and RGM-6 were exposed. He indicated he was preparing a summary, but we have not seen this review of the conditions.

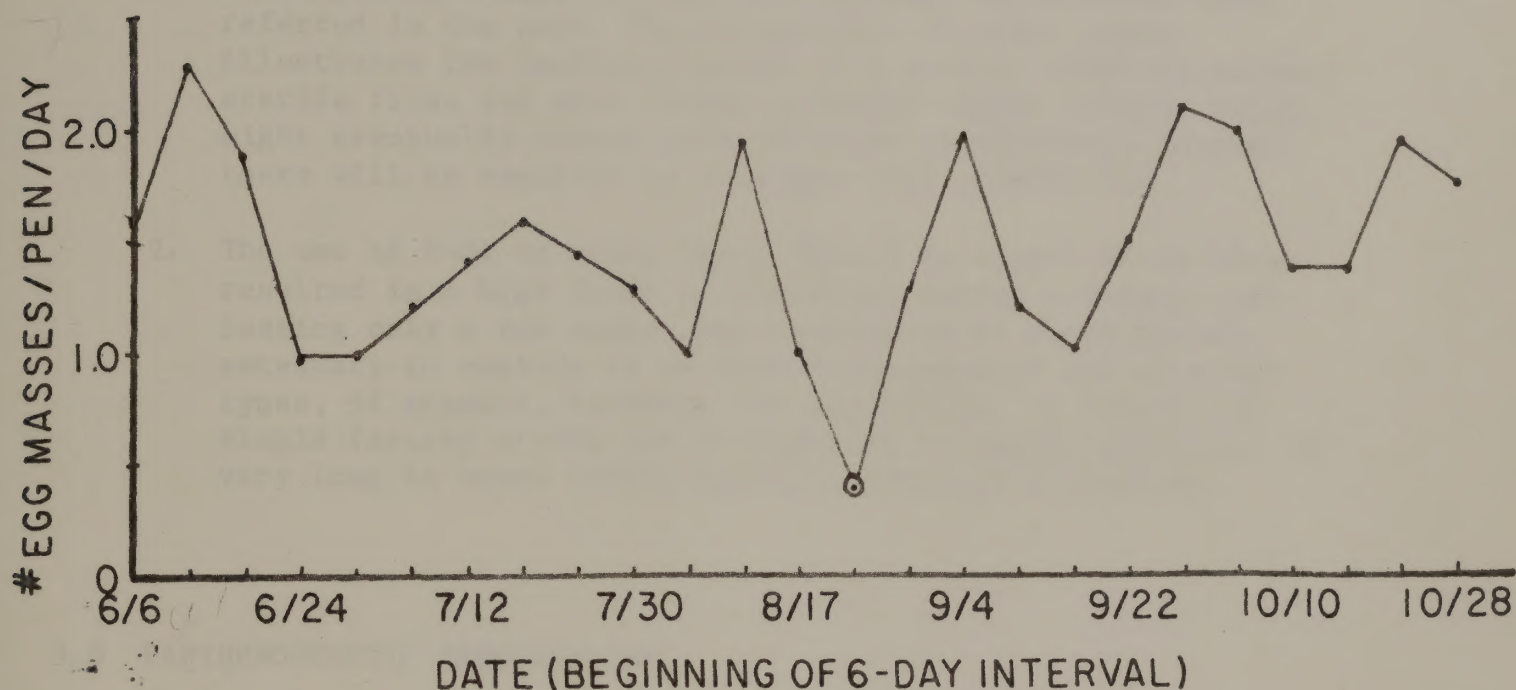


FIGURE 3. Average number of egg masses collected per sheep pen per day for 6-day intervals.

There is no general trend of either increasing or decreasing population size evident in this data -- although the circled point (8/23) represents an unusual collection. At that time there were newly wounded sheep, which are not as attractive as a rule, and there was continuous heavy rain. The typical collections ranging from 1 to 2 egg masses per pen per day returned immediately after these conditions changed.

Recently, Dr. Averhoff checked the eclosion of RGM-6 and it was over 95%. ARS staff are conducting a direct comparison of these two strains grown under the same conditions.

The original selection of RGM-6 was made after a few months had elapsed following its collection. Therefore there was considerable intrinsic screening for culturability, although the initial collection might have been larger had there been a major attempt to select a factory strain with optimum culture characteristics. Nevertheless, at the end of the test involving RGM-6 there was an excess of pupae obtained without special handling. It is unclear at this time whether the narrow based strain actually was less easy to culture than the broad based strain, but, if so, there is not enough difference to preclude use of narrow based strains in the factory.

2.7 Conclusions And Implications

1. Type-F was eliminated by a matching strain, RGM-6, and by a strain derived from a similar population near Veracruz, V-81. Type-D and Type-I increased in the presence of sterile flies, illustrating a type of "species release" to which we have referred in the past. The persistence of other types illustrates the ineffectiveness of a genetic mismatch between sterile flies and wild flies, although higher release rates might eventually reduce some of these populations. Further tests will be required to evaluate this possibility.
2. The use of V-81 in areas where Type-F is common would have resulted in a high level of sterility during a strain test lasting only a few weeks, but eventually it would become necessary to replace it as Type-F disappeared and as other types, if present, dominate the population. In effect, no single factory strain can be expected to remain effective for very long in areas having multiple types of screwworms.

3.0 PARTHENOGENETIC REPRODUCTION

The discovery of a case of probable parthenogenesis (asexual reproduction) suggests several questions that need immediate attention. These include:

1. Will parthenogenetic types undergo "release" as did Types-D and -I when the sexual types are removed?
2. Will an attractant lure parthenogenetic females to SWASS or traps?
3. Will parthenogenetic females lay enough fertile eggs to become a serious problem?
4. Is parthenogenesis in screwworms facultative or obligatory?
5. If parthenogenetic females were to become a serious problem not controllable by present methods, is there an alternative biological control available?

3.1 The LIC-3 Analysis -- (by John R. Ellison)

3.1.1 History -

The LIC-3 sibship was collected prior to the beginning of the Arriaga Test, on April 27, at the La Ilsa pen in zone C. This sibship was first indicated as unusual by Dr. McInnis (ARS) with his observation that a third chromosome was heterozygous. Analysis at U.T. of seven members of the sibship showed that 5 members of the sibship were normal, homozygous, Type F females, but two individuals were extraordinary in that they were triploid. These triploid individuals were further unusual in that they were composed of a diploid set of Type F chromosomes, plus a set of Type I chromosomes.

Other members of the sibship were observed when I visited the laboratory at Mission. Among the individuals available at Mission were male and female diploids that had both a haploid set of chromosomes from Type F and a haploid set from Type I. There were also one or more individuals at Mission that were homozygous Type F females.

3.1.2 Possible Origins -

There are several possible explanations for this very unusual sibship. I have listed these in the order of my evaluation of their probability for occurring, the most probable first.

1. The female parent was a Type F homozygote. She was able to produce Type F diploid eggs, as well as normal Type F haploid eggs.

The ability of a female to produce diploid and haploid eggs might occur in any of at least three ways. First, part of her gonadal tissue might have undergone somatic doubling to form autotetraploid tissue, which, when reduced by meiosis would produce diploid eggs. Second, the diploid could occur from a genetically controlled fusion of the second polar body nucleus with the egg nucleus during meiosis. Third, regular nondisjunction at meiosis 1 or meiosis 2 will produce diploid eggs. (The last two mechanisms could not occur 100% of the time, since haploid eggs occurred.)

This female was mated to a Type I male, which contributed the odd set of chromosomes in the triploids derived from fertilized diploid eggs. The heterozygous diploids resulted from fertilization of haploid eggs. Homozygous females would result from parthenogenetic development of unfertilized diploid eggs.

2. Another possibility is that the original female was triploid, Type F. Such a female could segregate on a regular basis both

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diploid eggs and haploid eggs. With fertilization as above, the progeny types seen could be generated. The problem here is that more than 90% of the progeny of a triploid female would be expected to be both aneuploid and lethal. It is doubtful that such a situation could have yielded sufficient offspring to survive culture.

3. One explanation of the events that occurred that does not involve parthenogenesis is that a homozygous Type F female could have had a somatic doubling of a portion of her gonad (or other method of producing a genetically diploid egg as well as haploid eggs as in 1 above), and mated SEQUENTIALLY with a Type F male and a Type I male. Having both Types of sperm, all observed types of diploid individuals could have been produced, and the triploids resulted from fertilization of Type F diploid eggs with Type I sperm. This explanation has the complication that successive matings appear not to occur in screwworms, and Type F triploids were not observed.

3.1.3 Highly Remote Possibility -

With reference to the Memo distributed by Dr. McInnis (undated, but about September 27, 1981) entitled "Analysis of LIC-3 Triploid Individual", I feel that his explanation is much less likely than those given above, and his cytological analysis suffers technical deficiencies.

His analysis is apparently only of chromosome length and centromere position, and does not concern itself with the subtle Q-band differences among the chromosomes. (These are the low contrast bands, not the extraordinary bands as appear on chromosome 3.) If one looks closely at the triploid karyotype (Figure 4), it becomes obvious that there is in each chromosome trio an odd chromosome which differs not only in length, but also in its fluorescence pattern. The Type I chromosome of each set is indicated with a * at its centromere in the drawing. It is also apparent that somatic pairing has situated the homologous chromosomes in close proximity, generally having a similar orientation. The Type I chromosome pairing with the 4th chromosomes of Type F would normally be classified as a chromosome 5. Likewise, the Type I chromosome pairing with the 5th chromosomes of Type F would normally be classified as a chromosome 4. Somatic pairing, however, indicates that the chromosomes that pair ARE homologous. Therefore all six chromosome sets are segregating.

With this information, I feel it is extremely unlikely that a female heterozygous for the X and chromosome 5, and a male heterozygous for chromosomes 3, 5, and 6 is likely to produce two, essentially identical, triploids. The presence of 6 homozygous Type F females in the sibship are even more unlikely with each parent segregating two or three

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chromosomes respectively, as the model would suggest. The situation becomes even more complex if one includes chromosome 2 and chromosome 4 as segregating entities. The probability of producing a single homozygous diploid Type F female from a parental cross in which (in one parent or the other) each of 6 chromosomes are segregating is $1/64$. However, the probability of producing 6 such homozygous females in a sample of 14 individuals is less than one chance in 25 million! This small probability becomes even smaller if one includes the probabilities necessary for the segregation of the triploids.

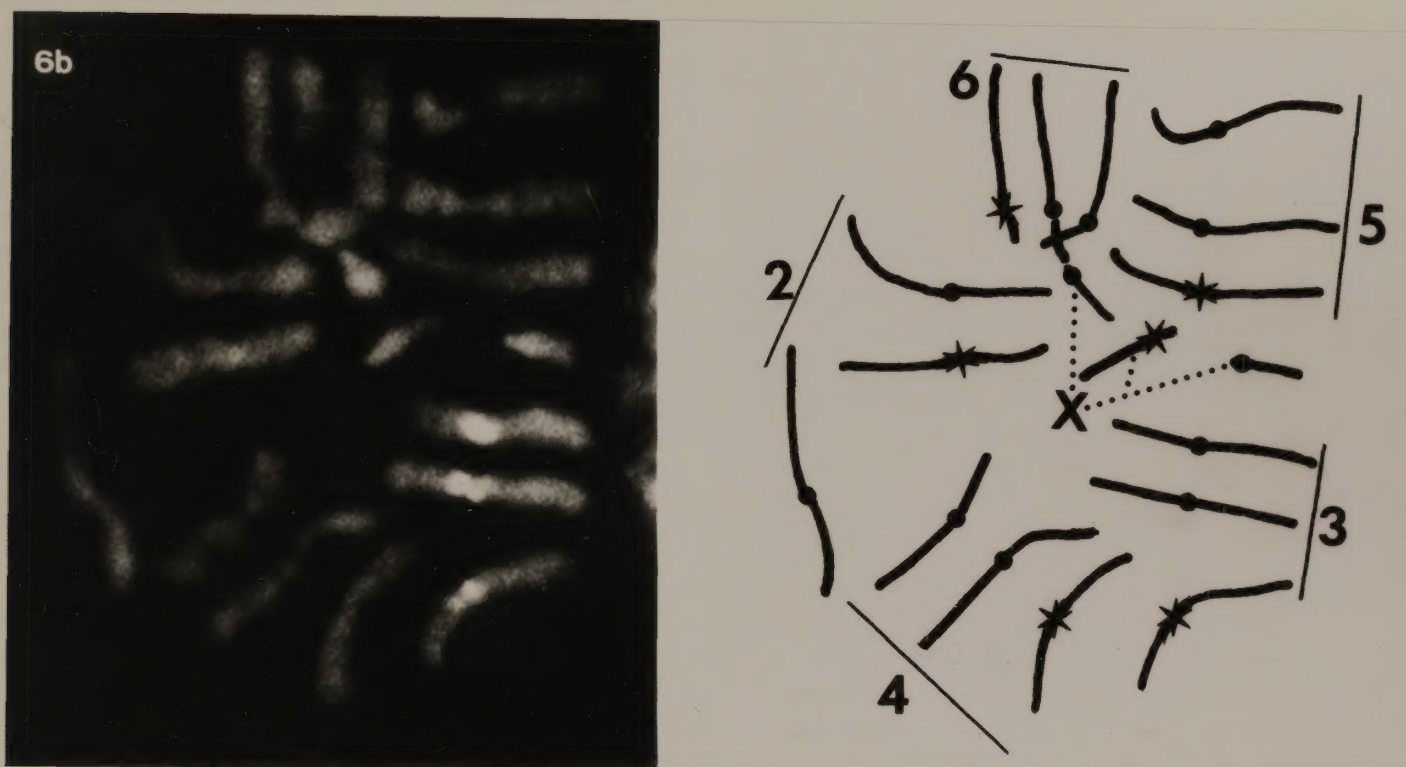


FIGURE 4. A micrograph of a triploid individual from an extraordinary wild hybrid sibship collected in Chiapas.

This is one of two triploid individuals that had two Type F chromosomes (indicated in the diagram by a dot at the centromeres) and one Type I chromosome (indicated by a star at the centromeres) for each of its 6 chromosomes. Chromosome 6 is not easily discernible in this micrograph, but chromosomes 2, 3, 4, and 5 and the three X chromosomes are clearly visible. Differences can be found in heterochromatic, quinacrine bright bands (chromosome 3), centromere placement (chromosomes 4 and 5) and in overall length (the X chromosomes and chromosome 4). In each set of three homologs, two of them match with respect to the low contrast Q-bands along the chromosomes, while the third member of the set is clearly not a match.

We have interpreted this individual as having arisen by the fertilization of a Type F, diploid egg with a Type I sperm. Other individuals seen in the same sibship included homozygous Type F females (6) which we have interpreted as probably arising parthenogenetically, and male and female heterozygotes that had one haploid set of Type F and one haploid set of Type I. The chromosomes are mitotically paired, and this pairing allows one to determine homologies in an unambiguous manner. A Type I chromosome is labeled here as chromosome 5 on the basis of somatic pairing with Type F chromosome 5, although it would be considered a chromosome 4 according to criteria used for chromosome identification. Likewise a chromosome 5 of Type I (here labeled as 4 I) pairs with the chromosome 4 of Type F. Thus the classification methods have resulted in an underestimate of the chromosome differences between these gamodemes.

Both Dr. McInnis and I agree that heterozygous individuals are very rare. The overall probability of this set of events occurring according to this model is the product of the segregation necessary for the diploids, the segregation necessary for the triploids, and the probability of appropriate heterozygous individuals mating. This product is so small as to make the model untenable as an explanation.

3.1.4 Conclusion -

My colleagues at the University of Texas are in complete agreement with the following conclusions. Although we certainly favor an explanation involving the parthenogenetic production of the diploid, Type F females, their true origin cannot be unambiguously determined from the data at hand. Nor is it certain that the apparent parthenogenesis seen is a regular, genetically controlled (and thus heritable) form of parthenogenesis.

3.2 Implications

At this time it is difficult to judge the potential impact of parthenogenetic reproduction on the eradication of screwworms. Much depends on the mode of inheritance, the distribution of the capability among gamodemes, the competitiveness of asexual females, the rigidity of the mode of reproduction, etc. Until cultures can be obtained for laboratory studies, little can be said with certainty. Nevertheless, many of these possibilities, should they prove to be real in screwworms, would profoundly influence the strategies of the program. We strongly

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urge the continuation of the screen for the presence of parthenogenetic types, and we recommend that all collections made in Mexico be so screened. We think the concern about parthenogenesis is justified because of the devastating impact such a system could have on the screwworm program should a parthenogenetic type become common.

4.0 NATURAL HYBRIDS

From the electrophoretic studies of enzyme variation it was found in the earlier studies that Type-I had an electromorph of phosphogluco-mutase (PGM) with a higher electrophoretic mobility (rf) than other types, although it shared the one most common among all types. In previous collections of Type-I from Arriaga we had not seen these faster moving electromorphs. In the collections from the Arriaga Test an unusual series of individuals, some heterozygous for this fast migrating PGM electromorph, were noted for egg mass LDA-23. Upon examination of the karyotypes of these individuals a natural hybrid with respect to chromosomes has been identified. However, it was an egg mass derived from a male with a karyotype differing from Type-I in the X-chromosome crossed to a typical Type-I female. A homozygous karyotype of this type may be found in LRC-96. Since the karyotype is similar in all chromosomes but the X and since the hybrid occurs in comparable frequencies to the homozygotes, it cannot be considered a new gamodeme. Therefore, this is a new type of X-chromosome included in Type-I gamodeme, and not an ~~intragamodemic~~ cross. In other words, the X-chromosome now is polymorphic in Type-I.

While this new karyotypic feature is extremely different from any others we have seen within gamodemes, and the biochemical diversity is considerable, it does not come close to meeting our criterion for defining a new gamodeme. It may be an incipient gamodeme, or an introduction of a geographic race of Type-I. Only further analysis will allow us to specify its taxonomic relationship to the "standard" Type-I.

5.0 ELECTROPHORETIC ANALYSIS

The primary use of electrophoretic data on enzyme variation has been to spot possible sibships of interest that either haven't been examined for chromosomal patterns, or might have features overlooked by the initial preliminary analysis. The case above concerning a natural hybrid is an example, and Type-J was first detected by electrophoretic patterns. However, the major time for examination of molecular variation is near at hand. This analysis requires a considerable body of data, and this was not available in an analyzable form until we had accumulated a

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number of sibships of known karyotype. It is not unlikely that we will see further subdivision of the gamodemes for genetic diversity, but, of course, there is no way of predicting if such units will be reproductively isolated to some degree, or be geographically isolated and have acquired a differentiated status with the extrinsic isolation. It will be the former that may be most relevant to the eradication program, although the latter could be useful in identifying possible origins of introductions, much like Dr. Milton Huettel (USDA/ARS, Gainesville) did with the Med-fly introduction in California.

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AGE DETERMINATION USING THE APODEME STRUCTURE

IN ADULT SCREWWORM FLIES (COCHLIOMYIA HOMINIVORAX)

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1.0 KEY WORDS

1. Cochliomyia hominivorax

2. Screwworm

3. Age determination

4. apodeme

5. cuticular bands

6. biological control

7. population biology

2.0 ABSTRACT

A method of age determination of adult screwworm flies is described and documented. This method uses the diurnal differences in the deposition of apodeme layers as a basis for counting the age in days since eclosion. The method is accurate to plus or minus one day, and can be determined on material that has been dried, pinned, frozen, or preserved in ethanol or other fixatives.

3.0 INTRODUCTION

A thorough understanding of the biology and natural history of any insect pest is necessary for its successful biological control. In the case of the screwworm fly (Cochliomyia hominivorax), information

concerning many of the parameters characterizing individuals in wild populations has thus far been unavailable or unreliable. In particular, there has been no accurate method for determination of the chronological age of adult flies.

The ability to determine the age of adult individuals collected from sentinel animals or SWORMLURE traps would contribute important information about longevity of wild flies, dispersion rate of flies from a primary infestation site, climatic effects and migration propensity. Knowledge of the chronological age of wild females in various reproductive states - virgin, non-virgin, nulliparous, parous, ovipositing - and the age of males at aggregation sites would elucidate the reproductive behavior in wild populations. Minimum, maximum and optimum ages at mating and oviposition could be determined.

The effects of sex and grouping on the longevity of flies in the laboratory has been studied (CRYSTAL, 1967) and studies on the effects of longevity on fertility of laboratory-raised flies have been carried out by DEVANEY and GARCIA (1975). Using laboratory flies, chronological age has been correlated with various physiological states such as flight activity of tethered flies (CRYSTAL, 1977), activity of the flight enzyme, α -glycerophosphate dehydrogenase (BROWN and SNOW, 1978), ovarian growth and development (LA CHANCE and BRUNS, 1963; ADAMS and REINECKE, 1979) and spermatogenesis (RIEMANN, 1967). GUILLOT et al. (1977 and 1978) used the reproductive status of the female and presence of pupal fat body to determine physiological age and as a means of estimating chronological age to characterize the reproductive structure of a wild population of flies. These studies, however, were based on studies of laboratory-reared flies and not on direct field observations.

Their relevance is therefore unknown.

A method resembling the procedure used by dendrologists in the ageing of trees by counting yearly growth rings has been used to determine the age of some insects. This involves counting daily growth bands in various cuticular structures. NEVILLE (1963) first described the presence of daily growth layers in the rubber-like cuticle, resilin, of locusts. Under UV light the layers autofluoresce brightly and dimly in an alternating pattern. As summarized by NEVILLE (1975) it appears that the autofluorescence is due to the formation of di- and tri-tyrosine crossbridges that crosslink the resilin as it forms. Higher temperatures increase the formation of crossbridges and thus the layers formed during the day have higher numbers of crossbridges and fluoresce more brightly than the layers formed at night.

NEVILLE (1967) was also the first to report the presence of daily banding on the apodemes of locusts. The apodemes are the internal cuticular projections of the exoskeleton on which muscles attach. Description of cuticular banding on apodemes of other insects has subsequently been reported. SCHLEIN and GRATZ (1972) determined the presence of daily growth rings in several Diptera, including Sarcophaga falculata, Calliphora erythrocephala, Glossina austeni, Culex pipiens, and Aedes aegypti. JOHNSTON and ELLISON (1981) described growth rings in Drosophila. TYNDALE-BISCOE and KITCHING (1974) used cuticular growth rings to determine age in field studies on the Australian sheep blowfly, Lucilia cuprina, and SCHLEIN (1979) used growth layers for age grouping of several anopheline malaria vectors in both laboratory and field studies.

Since the flight muscles increase in size after ecdysis in the locust or after eclosion from the pupa in the case of the Diptera, it is not surprising that the apodemes to which the muscles attach would also need to grow in order to provide firm attachment sites. KITCHING and ROBERTS (1975) demonstrated a very strong correlation between amount of thoracic musculature and the measured area for flight muscle attachment in the early adult life of the sheep blowfly, Lucilia cuprina.

These cuticular bands on the insect apodemes are visible in an ordinary light microscope. Their visibility does not depend on autofluorescence of tyrosine crossbridges. Indeed, tests performed by JOHNSTON and ELLISON (1981) for the presence of tyrosine in Drosophila apodemes were negative. Although the ultrastructure of these bands has not been determined (NEVILLE, 1975), it appears that the thickness of the cuticle plays a major role in their definition. Pigmentation may also be important. However, in the case of the Anopheles mosquitos and the Glossina specimens, the bands could not be seen without staining in hematoxylin (SCHLEIN, 1979). SCHLEIN (1979) attributed the bands to the day/night variation in deposition of this stainable material. In Drosophila, JOHNSTON and ELLISON (1981) have observed that the bands seem to be due to the thickness of the cuticle. In both Drosophila and Cochliomyia hominivorax the ability to detect these bands using the scanning electron microscope confirms that the thickness of the cuticle is the major contributor to the definition of the bands, since pigment deposition cannot be visualized using the SEM.

We have described the presence of cuticular banding at several locations in the adult screwworm fly, Cochliomyia hominivorax, and we propose that this banding can be used as a method for determining the

age of adult individuals in wild populations.

4.0 MATERIALS AND METHODS

All work was done using adult flies of known age that had been reared at the Screwworm Research Laboratory, Agricultural Research Service, USDA, Mission, Texas, and were shipped frozen in liquid nitrogen to the laboratories at the University of Texas at Austin. Preparation for both light microscope and scanning electron microscope will be described. The initial dissection and cleaning procedure is identical for both preparations but differs slightly between the thoracic apodeme and the ejaculatory apodeme.

4.1 Initial Dissection - Thoracic Apodeme (Third Furca)

The head and abdomen are carefully removed from the fly and the intact thorax placed in 10% KOH solution in a 13 x 100 mm test tube and heated to just below boiling (95 degrees C) in an aluminum block heater for approximately 10 minutes. This aids in removal of muscle and other tissue. Alternatively, submersion in 10% KOH at room temperature for approximately 24 hours is also effective.

The thorax is transferred to distilled water and, using fine forceps, the dorsal half of the thorax is carefully separated from the ventral portion and discarded. Muscle tissue is carefully removed. The third furca which is attached by a thin stalk to the metasternum is removed by breaking the stalk with fine forceps.

4.2 Initial Dissection - Ejaculatory Apodeme

The posterior 3 to 4 segments of the abdomen of a male fly is separated from the fly by means of a sharp razor blade and placed in a 10% KOH solution in a 13 x 100 mm test tube and heated to just below boiling (95 degrees C) for approximately 10 minutes, or overnight at room temperature.

The abdomen is then transferred to distilled water and the abdominal segments carefully removed to reveal the male genitalia. The ejaculatory apodeme is a fan-shaped structure which is attached by a transparent tube at the base of the aedeagus. It is easily separated from the aedeagus with fine forceps but care must be taken to prevent losing it in the tissue that surrounds the genitalia.

4.3 Preparation For Light Microscope

The structures (furca or ejaculatory apodeme) are dehydrated to absolute ethanol, cleared in xylene and mounted in silicon immersion oil (available from Olympus, I. F. 1.404). This oil was used as a mountant because its refractive index was such that Nomarski optics produced excellent contrast of banded apodemes even in the absence of pigment. The mount can be made permanent by ringing the preparation with fingernail polish. Light micrographs were made using a Zeiss photomicroscope with Nomarski differential interference optics and a 16X objective.

4.4 Preparation For Scanning Electron Microscope

The structures are dehydrated in a small amount of diethyl ether and mounted with Scotch double-stick tape on an SEM stob. The specimens are coated with a 25 micron gold/palladium layer using a Technics Hummer V sputter coater. Electron micrographs were made using an International Scientific Instruments (ISI) Super III-A SEM.

5.0 RESULTS

The locations of the screwworm cuticle where this banding can be seen most readily are on the third furca (the apodeme attached to the metasternum of the thorax) of both males and females and on the ejaculatory apodeme of the male. On the third furca, the rings are most clearly visible on the fan-shaped projection located on the anterior ventral surface. Figures 1 and 2 show the growth bands on the third furca of flies 6 and 15 days old. Figure 3 is the ejaculatory apodeme of a 15 day old male. Only bands that are continuous over a large area were counted as daily bands. In some cases it is difficult to exhibit the continuous line in a light micrograph because of focusing limitations, but they are readily seen on the microscope by focusing.

6.0 DISCUSSION

In all preparations made, including many not illustrated in this paper, one author (Hampton) would prepare the specimens and, without revealing the known age of the fly, present them to the other author (Ellison) for determination of age by counting bands. In a majority of

the preparations, the determination of age from counting the bands agreed exactly with the known age of the fly. In a minority of the cases the determination was within plus or minus one day.

Although growth bands could always be found on the ejaculatory apodemes, in some dissections it was not possible to find clear, countable growth bands on the thoracic apodemes. SCHLEIN (1979) and TYNDALE-BISCOE and KITCHING (1974) report that with laboratory-reared Anopheles mosquitos and Australian sheep blowfly, Lucilia cuprina, respectively, ageing of laboratory flies by growth rings was not always possible. However, when growth rings were detectable, in both insects, the age and number of bands were in agreement in laboratory specimens. It was found that for the screwworm apodeme, when bands were clear enough to be counted, agreement with known age was within plus or minus one day. Both SCHLEIN (1979) and TYNDALE-BISCOE and KITCHING (1974) report that the great majority of the insects collected in the field showed clear, countable rings. Since our research was limited to laboratory-raised flies it was not possible to confirm a similar phenomenon with screwworm apodemes.

In the case of Lucilia cuprina, TYNDALE-BISCOE and KITCHING (1974) showed that clear growth bands occurred in response to fluctuating temperatures in which the minimum temperature must fall below 15.5 degrees C and the maximum is at least 3.5 degrees C above this minimum threshold. SCHLEIN (1975) reported that intensity of color in growth bands in Sarcophaga depends on the amount of UV irradiation as well as ambient temperature. SCHLEIN and GRATZ (1973) found that variations in temperature in the laboratory improved the visibility of daily lines in Anopheles gambiae. Johnston and Ellison (1981) have observed that both

temperature and light conditions effect the nature of the depositions in Drosophila, but even in the absence of external environmental stimulus the bands are present.

The laboratory-raised flies that we used were reared under constant temperature (37 degrees C). Since quarantine regulations prohibit the raising of screwworm flies outside of special facilities, it was not possible to carry out experiments testing the effects of varying temperature and light. At the present time, laboratory flies are being raised in outdoor cages from the pupal stage, and collected and preserved on a daily basis by Dr. Wil Averhoff in Tuxtla Gutierrez, Chiapas, Mexico. These flies should provide information on the effect of natural variation of temperature and day length on the formation of rings. If temperature fluctuation is important in the deposition of the bands in the screwworm fly it would be expected that wild-caught flies would show a more distinct banding pattern. ✓

Among the advantages offered by the method of counting of cuticular bands for age determination are:

1. The method of preparation is relatively fast and simple and the bands can be counted using a compound microscope with no special optics. Nomarski differential optics enhances the banding, but it is not necessary to detect it.
2. Cuticular banding gives estimates of chronological age; whereas, pupal fat body presence can determine only relative age (young and old) and ovarian development although correlated with a range of actual age in laboratory-raised flies can still only give a relative estimate. Also, environmental factors

such as availability of food for adults, availability of oviposition sites, and climatic conditions may affect ovarian development. TYNDALE-BISCOE and KITCHING (1974) report that protein deficiency in the diet of female flies of Lucilia cuprina can arrest ovarian development. Thus, age based on ovarian development under such circumstances would produce an underestimate of the flies' ages.

3. Cuticular banding appears to be the only effective way to age males. Analysis of the reproductive state of the male would be of limited value since, according to RIEMANN (1967) mature sperm are found in males as early as 1 day old. The basal sac of the testes where sperm are stored reaches its approximate maximum volume by age 4 days and after that time the quantity of sperm does not increase much even in unmated males.

Cuticular banding, offers two opportunities, the third furca and the ejaculatory apodeme, for determining age in males.

Cuticular banding of apodemes seems to be common to many insects and as such may prove useful for both research and pest control applications. The methods presented are generally useful for many genera of insects. This laboratory has observed such bands in several species of ants, the Med-fly (Ceratitus capitata), house flies and several species of *Drosophila*. From the data at hand, the method seems to be accurate to within a day. The accuracy is improved by the availability of several sites within a single individual from which independent determinations can be made.

7.0 LEGENDS TO FIGURES

1. The third furca of a 6-day old screwworm adult. Note that the banding pattern can be observed in several different parts of the apodeme. Light micrograph, Nomarski, 380X.
2. The third furca of a 15-day old screwworm adult. Note that the banding becomes more narrow as the fly ages, but is clearly discernable at 15 days. Light micrograph, Nomarski, 380X.
3. The ejaculatory apodeme of a 15-day old male adult. There are many smaller bands appearing between major bands at various places in the apodeme. The ejaculatory apodeme offers the most conspicuous banding of any apodeme. The first band is considerably wider than others. The apodeme seems to grow very rapidly for the first 3 days after eclosure. SEM, backscatter imaging system, 400X.

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FIGURE 1

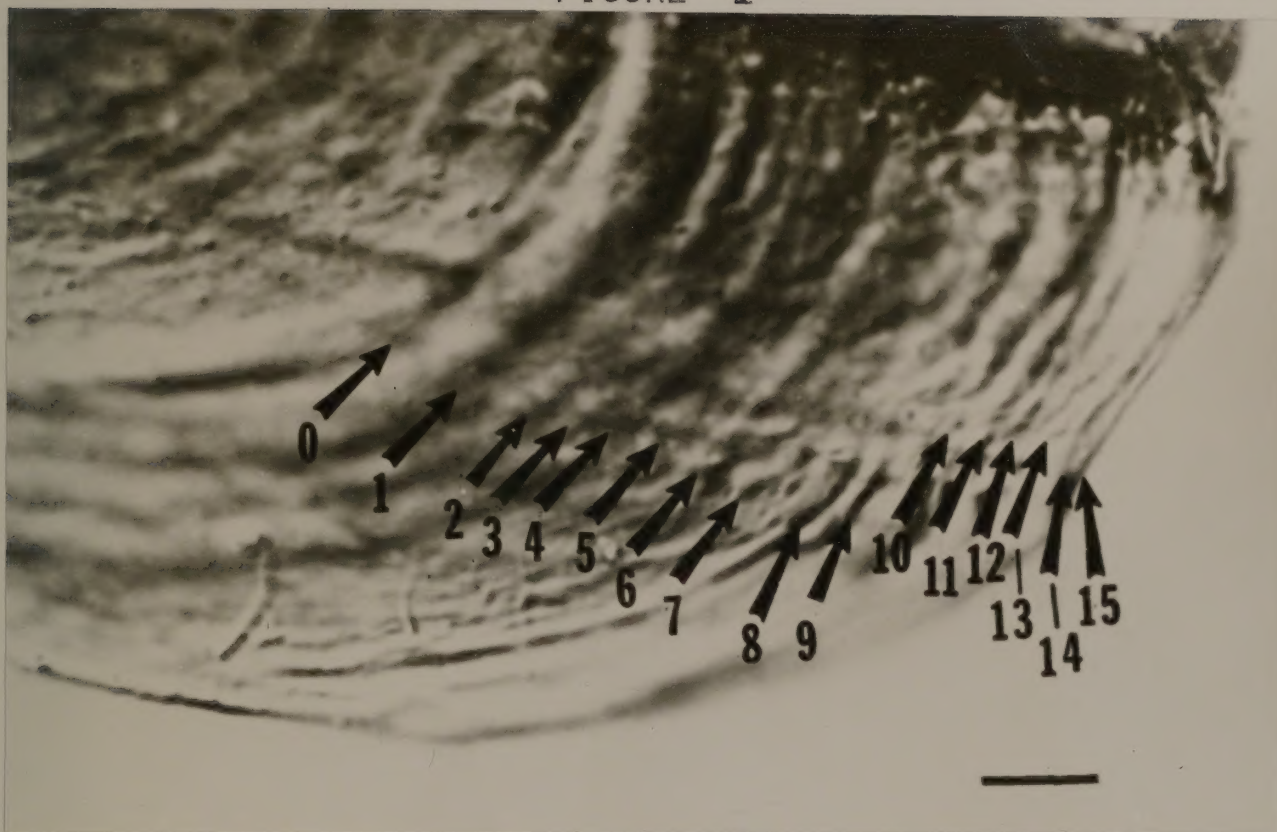
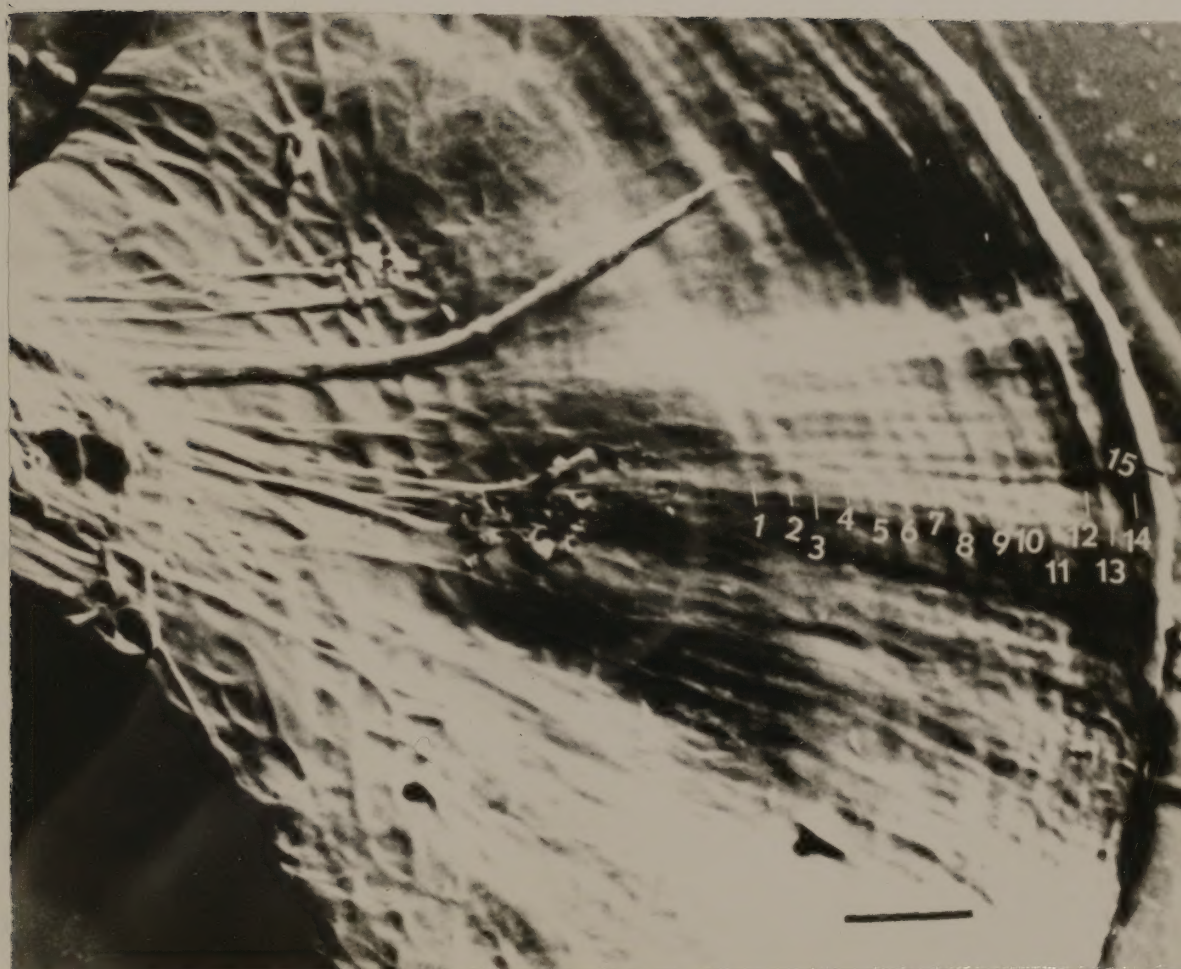


FIGURE 2



FIGURE 3



9.0 REFERENCES

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